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# THE SPERMATOGENESIS OF SCOLOPENDRA HEROS.

BY MAULSBY W. BLACKMAN.

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# *The Spermatogenesis of the Myriapods.*

## *III. The Spermatogenesis of Scolopendra heros.*

By Maulsby W. Blackman.

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## I. Introduction.

The investigations upon which this paper is based were begun during the winter of 1900–1901 in the Laboratory of Zoölogy and Histology of Kansas University, and were completed in the Zoölogical Laboratory of Harvard University during the present year (1904–1905). The greater part of the observations on the spermatocyte changes of Scolopendra heros were made while working at Kansas University, although

many of the figures were later redrawn and a number of additional observations made. Practically all of the work upon the metamorphosis of the spermatid was done in the Harvard laboratory.

It is with pleasure that I take this opportunity of expressing my gratitude to Dr. E. L. Mark for much valuable advice and counsel in the work upon Scolopendra, and for his careful reading and criticism of my paper. My thanks are also due to Dr. C. E. McClung for his advice and encouragement during the early part of the work.

Comparatively little cytological work has ever been done upon the myriapods, probably owing to the difficulty of obtaining material, in the required stages, of the more common members (Geophilus, Julus) of this group. But such difficulties are not experienced in working upon the various species of Lithobius, Scutigera, and Scolopendra. This apparent neglect is certainly not due to any lack of excellence in the material, for while the chromosomes are not as large as in the sperm cells of some insects (Acrididae), they are still of such size that their behavior may be followed in the minutest detail, and they are in some respects more favorable than those of other arthropods. Then, too, in the study of other problems than those depending on the size and number of the chromosomes, — such as the behavior of the cytoplasmic structures and the evolution of the spermatid into the spermatozoön, — the cells of Scolopendra are decidedly superior to those of any other arthropod I have examined.

The published works upon the spermatogenesis of myriapods may be divided into two groups: the first comprising the early works by Gilson ('84), Carnoy ('85), and Prenant ('87); the second including the later works by P. Bouin (:00, :01, :03), P. et M. Bouin ('99, :02, :03), Bouin et Collin (:01), Collin (:01), Meves und von Korff (:01), and Blackman (:01, :03).

The early work of Carnoy ('85) upon the spermatocytes of Lithobius forficatus, Geophilus, Scutigera arachnoides, and Scolopendra dalmatica, while in many respects valuable, contains numerous inaccuracies, as do also the observations of Gilson ('84) and Prenant ('87). These mistakes, which in many cases are doubtless due to the imperfect technique of the time, have at several points been the cause of errors of interpretation. The results of these authors will be discussed later in connection with the observations upon which they have a bearing.

In 1901 appeared a short paper by Meves und von Korff upon mitosis in Lithobius. Their observations are concerned principally with the

formation of the spindle and its peculiar modification in the division of the first spermatocyte. During the early prophase the centrosomes never rest directly upon the nuclear membrane, but remain at a considerable distance from it, and in the later prophase they migrate in opposite directions, until they come to lie very close to the cell membrane. From these proceed the astral rays, none of which seem to connect in later stages with the spindle fibres. The linin of the nucleus gives rise to the fibres of the spindle, which, though the poles are truncate, are directed respectively toward the two (now double) centrosomes, a condition that is often seen in plant cells. The astral fibres radiating from the centrosomes remain entirely distinct from those of the spindle. Concerning the origin of the chromatin the authors have little to say, since in the earliest stages studied by them the chromosomes were already in the form of distinct elements. At this stage the "nucleolus" has already broken up into several fragments, which are colored red by the Ehrlich-Biondi stain. I should like to mention in this connection that in preparations of three species of Lithobius collected at three widely separated localities, Kansas, Massachusetts, and Bermuda, I have observed that the chromosomes arise from the nucleolus-like body or karyosphere. This is shown in material fixed by various reagents and stained by several methods, including Heidenhain's haematoxylin and the Ehrlich-Biondi triple stain. In a later paper I hope to discuss at length the origin of the chromosomes.

In a series of short papers from the University of Nancy, P. Bouin, M. Bouin, and R. Collin have dealt with various problems connected with the spermatogenesis of Lithobius, Geophilus, and Scolopendra. The first of these (P. et M. Bouin, '**99**) is concerned with the presence and evolution of certain irregularly formed bodies in the cytoplasm. These the brothers Bouin believe to arise by the breaking down of the astral fibres; later they undergo a sort of gelatinous metamorphosis, and in the early prophase disappear. Meves und von Korff (:**01**, p. 482), however, find these bodies throughout the whole period of mitosis of the first spermatocyte, although during the later stages they break up into smaller granules.

In several later papers Bouin and his colleagues have described modifications of the spindle in several species of myriapods — Lithobius forficatus, Geophilus linearis, and Scolopendra morsitans — similar to those described by Meves und von Korff. Other results of these authors will be mentioned later at various places in this paper.

## II. Material and Technique.

The material upon which these observations were made was obtained from Scolopendra heros, the large centipede most common in the southwestern part of the United States. The greater part of the work was done upon material obtained from Russel County, Kansas, in June, 1900, through the kindness of Mr. W. S. Sutton. Later a number of specimens of the same variety of S. heros were received from Beulah, Colorado, through Mr. R. E. Scammon.

The testis of S. heros consists of a variable number of follicles lying near the dorsal wall of the body-cavity and communicating with the exterior at the hind end of the body by means of the vasa deferentia. Usually the follicles are united in pairs to constitute a lobe, but occasionally there is only a single follicle to a lobe. Each follicle is in effect a blind tube, its only connection being with the vas deferens.

The arrangement of the various cell generations within the follicle is quite different from that existing in insects. This would naturally be expected in view of the fact that the centipede is a perennial animal and that the testicular elements must therefore be so arranged as to permit the annual regeneration of the organ. The extreme periphery of the follicle is occupied by spermatogonia in various stages of so-called rest and of cell division (Plate 1, Fig. 1). In the mature testis these never form a continuous layer. From the periphery to the centre of the follicle, there are found in the order given: (1) young spermatocytes, of many sizes and stages of growth, (2) spermatocytes in the "vesicle" stage and in various phases of the maturation divisions, (3) spermatids in different stages of metamorphosis, and (4), in the centre of the follicle, mature spermatozoa.

In a series of preparations made in June, about one half of the volume of the follicle is occupied by spermatozoa. In preparations of material, captured in August and September, the follicles are smaller and the space occupied by spermatozoa is relatively somewhat less. It is worthy of note that the follicles of the testis have not all developed at the same rate, so that in the ripe testis all stages are abundantly represented.

In the preparation of the material two fixing fluids were used: Flemming's chromic-osmic-acetic mixture and Gilson's nitric-acetic-sublimate mixture. The results with Flemming's fluid, while fairly good, were so inferior to those obtained with Gilson's mixture, that in the later preparations the latter reagent was used exclusively. With Gilson's fluid

the fixation in favorable material is apparently perfect. There is no perceptible shrinkage, and at least the grosser structure of the cells is identical with that observed in living cells derived from the same source.

The material was left in this killing fluid for lengths of time varying from twenty-four to sixty hours. Probably the best results were obtained with material fixed forty-eight hours, although there is little apparent difference due to variations in the length of exposure. It is always well to use a large amount of fluid, thirty to fifty times the volume of the object, and if left for more than twenty-four hours in the fixing reagent, this should be renewed. After fixation the objects were washed for several hours in running water and then gradually dehydrated in alcohol of the grades of thirty per cent, fifty per cent, and seventy per cent. To remove the sublimate and prevent the formation of crystals, a few drops of an alcoholic solution of iodine were added to the seventy per cent alcohol.

In order to obtain the best results with material fixed in Gilson's mixture, I have found that it is necessary to employ for infiltrating and embedding, the combined celloidin and paraffin method described in a former paper (Blackman, :01, p. 62). While this fixation if properly carried out is nearly perfect, it leaves the tissues very soft, so that, if the ordinary paraffin method is employed, some shrinkage must inevitably occur. With the celloidin-paraffin method this disadvantage is obviated.

The sections were cut with the Minot precision microtome, the thickness varying from 2 to 6 micra, and were affixed to the slide in the ordinary manner by using very dilute albumen water (two drops of Mayer's albumen to one ounce of distilled water). Then the paraffin was removed by means of xylol and the sections stained as noted later. In the finer work it was found desirable to remove the celloidin also. This may be done, either before or after staining, by placing the slide for a few minutes in a mixture of absolute alcohol and ether.

In staining the sections a number of methods were used. The drawings and photomicrographs accompanying this paper were made exclusively from sections stained in Heidenhain's haematoxylin, either used alone or in conjunction with Congo red. For microchemical tests, Bismark brown, cyanin, methyl green, Auerbach's methyl-green-acid-fuchsin, Flemming's three-color stain, and numerous other dyes were used. For the study of the chromatin structures the preparations stained with Heidenhain's haematoxylin were the most favorable, although in some

cases counterstaining with Congo red was of value. However, the chief value of the latter stain was found in the work upon the metamorphosis of the spermatid, where it was nearly indispensable in studying the early stages in the formation of the axial filament. For in sections stained with haematoxylin alone the very young axial filament could hardly be distinguished from the cytoplasm, but when Congo red was used as a counter stain, the cytoplasm in well-decolorized sections was stained orange-red and thus served as an excellent background for the black filament. I believe that the value of Congo red as a counter stain for haematoxylin, when used after Gilson's fluid or other sublimate-acetic mixture in the study of the spermatid changes, can hardly be overrated.

## III. Observations.

### 1. Introductory.

In studying the spermatogenesis of Scolopendra one is surprised at the striking similarity of the cells in their general appearance and in their behavior to those of the female germ elements of various animals. Indeed, it is often the case that, were the cells isolated and mounted by themselves, they would be immediately and invariably mistaken for stages in oögenesis. This resemblance is most striking in the stages beginning immediately before the prophase of the first spermatocyte and extending to the completion of the second spermatocyte, but it is also very pronounced even in the earlier phases.

After the completion of the "division period" the cells, which are at that time very small, immediately begin to increase rapidly in size. They continue to grow until finally, when they are ready for the maturation divisions, their bulk is many times that of the spermatogonium. This enormous growth suggests of itself a comparison of the spermatocytes of Scolopendra with egg cells. But there is also a surprising similarity in the structure of the cells themselves as well as in their general appearance. By the time the two cells arising from the last spermatogonial division are really separated by a membrane and the nucleus has been reconstructed, the cytosome has already increased considerably in size. The chromatin at this stage, as in eggs, is in the form of a number of granular segments distributed irregularly throughout the nuclear space, and upon one side of the nucleus, in close contact with the membrane, there is a chromatin body which in general shape resembles the karyosomes often seen in growing eggs. In later stages the

spermatocyte resembles the egg cell still more. It has become much enlarged, and by its growth the cytosome has increased greatly, and out of proportion to the nucleus. Thus, even in size, the spermatocyte at the completion of the growth period resembles the egg. The elements within the nucleus have become so arranged that this space is now occupied by a very fine reticulum of granular linin, which is no more dense than the cytoplasm outside the nucleus and stains in a similar manner. All the chromatin has apparently been withdrawn from this network and is now contained in a large, peripherally placed, nucleolus-like body, which stains in such a manner as to leave no doubt whatever as to its richness in chromatin. From the striking resemblance of the cells at this time to egg cells during the stage of the germinative vesicle I have called this the pseudo-germinal vesicle stage of the spermatocyte, but shall in future call it simply the "vesicle" stage.

During all the growth period the centrosomes can generally be found in these cells and can, indeed, be identified at all stages from the prophase of the last spermatogonium up to the formation of the spermatozoön. During the growth period they are contained in a centrosphere of slightly modified archoplasm, which is often readily distinguishable from the remaining archoplasm of the cell. Throughout the following division the chromatic and archoplasmic structures behave in a manner similar to that often recorded of egg cells during the maturation and cleavage stages. Indeed, the division figures resemble those characteristic of cleavage stages much more closely than they do the maturation mitoses of eggs. This is of course to be expected, since here there is to be an equal division of the cytoplasm.

During the changes following the beginning of the growth period there arise what are apparently two types of spermatocytes. These are probably produced by differences in the environmental conditions surrounding the different cells. That they result in the formation of fundamentally different kinds of spermatozoa, is very improbable when we consider their later behavior. They, however, show notable differences at various stages, and it is therefore convenient to recognize the two kinds. The cells of the larger type resemble egg cells much more closely than do those of the small type. This is true both as regards general appearance and specific behavior.

Not only are the division figures of these spermatocytes very similar to those of the female germ cell, but the cells arising by these divisions are also at first very similar. The nuclei of the second spermatocyte and the spermatids are peculiarly like the female pronucleus and the

later cleavage nucleus of the egg. They are comparatively small vesicles in which the chromosomes are closely aggregated and in which the amount of linin is much smaller than in the "vesicle" stage. Thus we see that during all the spermatocyte stages the cells exhibit phenomena similar to those characteristic of the female germ cell undergoing maturation and cleavage.

What is the explanation of this resemblance? In my description of the structure of the testis I have given in a general way the position of the parts and the conditions surrounding the cells at various stages. I will repeat this description here and see what conclusions can be drawn from it. The testis is divided into a number of follicles, each one of which is connected to the vas deferens by a tube which is continuous with the wall of the follicle at one end (Fig. 1). The lumen of this duct is continuous with that of the follicle, which extends nearly the entire length of this structure and is filled with spermatozoa. Upon the extreme periphery of each follicle are arranged the spermatogonia, either in the resting stage or in various phases of mitosis. Next to these are disposed in sequence from periphery to centre: (1) spermatocytes in various stages of growth; (2) first spermatocyte in the vesicle stage and in various phases of the first maturation division; (3) second spermatocytes in various stages; (4) spermatids; (5) young spermatozoa; and (6), in the lumen, mature spermatozoa.

These different cells in various stages are distributed in more or less regular layers. There are, to be sure, many irregularities in this distribution, the most pronounced appearing in follicles which are far advanced in development. Here the closely packed spermatozoa fill the lumen and show a tendency near the middle of the follicle to encroach upon the younger cells, so that these are frequently forced aside, and the mass of spermatozoa thus comes more or less close to the follicular sheath (Fig. 1). However, the most typical arrangement is that which I have mentioned. This is especially marked for the larger type of spermatocytes, which, as I shall show later, are always arranged in a definite manner in the follicle.

In the interstices between the cells, there is present at all stages a substance of a more or less viscid consistency, which very probably serves as nourishment. In testes which have been fixed this material consists of a basis which in general structure very much resembles cytoplasm. This reticular substance is not, however, of the same nature as cytoplasm, its reaction to various stains being different. It does not show a strong affinity for iron-haematoxylin nor for other similar stains, but is colored

deeply by Congo red. In the interstices of this reticular matrix of the testis are contained numerous granules and globules of nutritive material. There are at least two kinds: (1) globules of an oil-like appearance, which in the material fixed in Flemming's mixture are stained in the manner which is characteristic of oil droplets under the action of osmic acid; (2) numerous irregular masses of a granular consistency, which react in a different manner to reagents. This material is not colored by the fixing reagents, but when stained with iron-haematoxylin and Congo red assumes a brown color. While this interstitial substance is present around all the cells, it is not equally abundant in all regions. At the periphery of the testis, around the spermatogonia, there is not nearly as much as in the deeper layers, in the region of the growing spermatocytes; and here there is not as much as in the large intercellular spaces enclosing the larger type of vesicles. Surrounding these immense cells there is often quite a thick layer of this nourishing matrix. The cells of the smaller type of spermatocytes are not so well supplied with this food material as the large ones are; the results of this I shall discuss later, when speaking especially of these cells. There seems to be a greater pressure in the region occupied by these smaller cells, which forces them closer together and thus diminishes the supply of nourishment which they receive.

Thus the spermatocytes of Scolopendra are supplied during the growth period with large quantities of food material, much more than is usual in the case of the testis cells of other animals. Indeed, the conditions very closely approximate those existing around the egg during the corresponding stages of its evolution. This is especially marked for the large spermatocytes, — the type which more closely resembles the egg in structure and behavior, — but is also true to a less degree of the smaller type of cells.

These facts help to explain why the spermatocytes of Scolopendra resemble so closely typical egg cells. The conditions surrounding the cells of the testis are practically identical with those of the ordinary egg. Nourishment is accomplished in a similar manner. The arrangement of the large spermatocytes in the follicle is similar to that of the eggs of many animals.

From observations given more in detail in the course of this paper, I believe we are justified in concluding that the germ cells of this animal are in a very plastic condition. They are but slightly differentiated and are hence very easily acted upon by environmental influences. Because of a very plentiful supply of nourishment these cells increase greatly in

size and come to resemble eggs, and, because the supply of nourishment to be obtained by some cells is more than that accessible to others, there arise two types of cells, which, as long as this different condition endures, exhibit considerable differences in their behavior. When I first observed these two types, their apparently marked differences at certain stages led me to believe that they must have arisen from cells which were radically different in preceding generations, but upon more careful study this was found not to be the case. There being but one type of spermatogonium, I am led to believe, from extensive observations, that the differentiation arises as a secondary characteristic in the spermatocyte stages alone. The late spermatogonia and the early spermatocytes exhibit no such divergence whatever. Study of later stages also reveals the fact that the very evident differences of the two kinds of cells are not so important as was at first supposed. For in their later behavior, in the second spermatocyte and in the spermatid, the cells of the two types again exhibit practically identical phenomena. It is very interesting to note in this connection that the slight differences in the environmental conditions characteristic of the large and small types of spermatocytes at the earlier stages, no longer exist in the later ones. The relation of the cells to their food supply is now practically identical in the two types, and as a consequence they behave in a manner as nearly alike as is possible in view of the marked difference in size and the slight variation in structure. From these facts it seems to me very improbable that these two types of cells develop into essentially different kinds of spermatozoa.

### 2. Spermatogonia and Early Spermatocytes.

The spermatogonia of Scolopendra heros are rather small cells, which in the well-developed material principally used in these observations are confined exclusively to the extreme periphery of the testicular follicles (Fig. 1; Plate **8**, Fig. 123). In shape they are oval, the long diameter, which is several times greater than the short, being parallel to the long axis of the follicle. The cells of the earlier stages are often in such very close contact with the fibrous sheath of the testis that it is difficult to study them carefully. However, occasionally there are to be found groups of a dozen or more (Fig. 123) which project out into the mass of young spermatocytes, and it was mainly upon these that the observations of the earlier stages were made.

In what may be called the resting stage of the spermatogonia (Plate **2**, Fig. 2) the nucleus, which fills a large part of the cell, is an oval body sur-

rounded by a membrane much more sharply marked than that enclosing the cell itself. It contains within it a large nucleolus-like body (karyosphere) and a fine dense network of faintly staining fibres. The whole cell stains very feebly with haematoxylin and the other stains employed, the nuclear network being no more conspicuous in this respect than the cytoplasm. The karyosphere, however, stains very deeply, and thus shows, even at this early stage, its chromatic nature, although it takes the stain less readily than in the spermatocytes. When decolorizing is continued long enough, it is plainly seen that this element is not homogeneous, but is very finely reticulated. It is also evident that it gives up its stain much more readily than is the case in the spermatocyte, which shows, I believe, that there is more linin and less chromatin present in this body in the spermatogonium than in the spermatocyte.

I have never observed a centrosome at this stage, but as the number of cells studied is comparatively small, it may have been overlooked. Indeed, when the fact is mentioned that from the early prophase of the ensuing division up to the maturing of the spermatozoön, centrosomes can be readily identified, it seems probable that they are present also in the spermatogonia even at a time when these cells are not actively dividing. In the succeeding prophase (Fig. 3) the cell undergoes the following changes: The cell outline becomes more rounded and the cytoplasm appears more transparent. There are now plainly to be seen in the vicinity of the nucleus two small but very distinct centrosomes. These at first seem to lie free in the cytoplasm and are not surrounded by a sphere nor by radiations. Later very faint radiations appear, thus indicating the nature of these granules. The nucleus has also changed considerably (Fig. 3) and has assumed a more nearly spherical outline. The karyosphere has disappeared, and the chromatin derived from it has taken the form of thirty-three small chromosomes. All of these, with the exception of one, are irregular in outline and of a granular appearance. This one, the accessory chromosome, possesses a clear-cut outline and is apparently homogeneous in structure. From the phenomena observed in the spermatocyte later, it is probable that in the preceding resting stage this element formed the centre about which the rest of the chromotin of the cell was aggregated to form the karyosphere described above.

In succeeding stages the centrosomes separate and move to opposite poles of the nucleus, the nuclear membrane disappears, and the mitotic figure is formed as usual. In the metaphase (Fig. 4; Plate 8, Fig. 125) the chromosomes become irregularly arranged in the equatorial plane and

often are so closely crowded in the narrow limits of the cell that the individual elements cannot be distinguished.

The archoplasmic structures at this stage are well developed for such small cells. The centrosomes are minute, but very distinct, dots, about which the cytoplasm is slightly more dense, forming rudimentary centrospheres. The spindle fibres are numerous — apparently more so than the chromosomes — and take a dark stain. The archoplasm is arranged about the centrosomes in a radiate manner, forming faint astral rays.

In later stages the cell begins to elongate, the lengthening of the cell being accompanied by a corresponding change in the form of the spindle (Fig. 5). Accompanying this elongation of the spindle, the chromosomes are divided and immediately drawn toward the poles (Fig. 5). When this takes place the interzonal filaments, as usual, are seen extending between the separating groups of chromosomes. This is not strange in view of their persistence and marked behavior in the succeeding stages. In Figure 6 is shown an oblique polar view of a cell in the late anaphase. The chromosomes are grouped in a closely packed mass at the pole, and the spindle fibres present the appearance of radiations of the cytoplasm centering in this chromatin mass.

When the divided chromosomes have become aggregated in dense masses at opposite poles (Fig. 5), the cell lengthens still more and immediately begins to constrict. The constriction is never entirely completed at this time, for, near the centre of the wall between the two cells, there always remains a small round opening (Figs. 7–12). By this constriction of the cell the interzonal filaments are forced together into a sheaf-like bundle extending through the opening in the constricting wall and with one extremity in each cell. Immediately after the separation of the chromatin has been accomplished, the centrosome divides into two small bodies, and at the stage represented in Figure 7, these have become surrounded by a mass of archoplasm. This is of a very finely granular consistency, and undoubtedly is formed by the breaking down of the astral rays and the polar ends of the spindle fibres.

When the chromosomes migrate to the poles of the cell, they are grouped into such a dense mass that the outlines of the individual elements cannot be distinguished. Soon, however, this mass begins to show signs of important change. Processes arise from the surface, and soon (Figs. 7, 8) the outlines of many of the individual elements can be distinguished. It is apparent that they are lengthening and becoming diffuse and granular. At places, however, they are still so closely grouped that individual outlines cannot be traced. One of these

elements, however, does not assume a granular condition, but retains all the characteristics of a chromosome in the metaphase. This is the accessory chromosome. The whole mass of chromosomes at this stage is surrounded by a clear space irregularly bordered by the cytoplasm, but possessing no membrane. Figures 7, 8, represent a later stage in the disintegration of the chromosomes. The chromatin threads are still more granular and have become elongated into much more slender filaments. The accessory chromosome is still intact, and is plainly to be distinguished in the loose mass of granular filaments. The nuclear membrane is not yet re-formed. This figure and the one following resemble very much the synapsis figures of Moore ('95) for elasmobranchs, and those of Montgomery ('98[a]) for Pentatoma. In the material of both these investigators the synapsis, or fusing together of the chromosomes into pairs, is said to be accompanied by an aggregation of the chromatin threads at one side of the nucleus. In both, the pseudo-reduction apparently takes place considerably later than in Scolopendra, viz., after the reconstruction of the nuclear membrane and the formation of the spireme. In Scolopendra there is no true spireme stage, — i. e., a stage in which all the chromosomes are united into one long thread, — unless, indeed, such a condition is present when the chromatin is aggregated in the karyosphere during the vesicle stage; but this seems very unlikely when we consider the subsequent behavior of the chromatin during the prophase of the first spermatocyte. This stage (Figs. 7–10) in Scolopendra is the synapsis stage in the correct sense of the term. The chromosomes in the anaphase preceding are of the somatic number, and in the stages immediately following (Fig. 10) are of the reduced number. During the entire synapsis, which begins in the early telophase of the last spermatogonium (Fig. 7), the chromosomes are aggregated into a more or less dense mass. On account of this close massing and of the small size of the cells at this stage it is impossible in S. heros to follow the details of this process as Sutton (:02) has done in Brachystola magna. However, in the stages immediately succeeding synapsis the appearances both in the present species and in S. subspinipes leave no doubt as to the truth of the statement that reduction is accomplished by an end to end union in pairs of spermatogonial chromosomes (Fig. 11). Such stages are much more common in the latter species, where they were first observed, but they also occur in S. heros. At this stage each of the chromatin segments, which are of the reduced number, is composed of two equal or approximately equal parts joined together. At the point of junction there is a small space, bridged ap-

parently by linin strands, where no chromatin occurs, and the filament is often bent at this point into a V-shaped figure.

The accessory chromosome apparently takes no part whatever in this process, but retains all of the characteristics previously noted for it. It is included within the mass of chromatin filaments, or is in very close approximation to this, but undoubtedly receives no addition to its substance except that obtained by ordinary growth. From the foregoing it is evident that the total number of chromosomes is not reduced exactly one half, since the reduction does not affect the accessory chromosome.

The next change in the condition of the chromatin is represented in Figures 10, 11. Here the synapsis is completed. The chromosomes have withdrawn from the side of the nucleus and have become irregularly arranged throughout the nuclear space, and the accessory chromosome has assumed the peripheral position which is characteristic of it in all arthropod spermatocytes. It is now readily seen that the chromatic segments are fewer than in the spermatogonia, and by careful counting it is ascertained that they are present in the reduced or spermatocyte number. This stage is as near the spireme stage as any that I have observed in Scolopendra. As I have shown in a previous paper (Blackman, : 03), however, there is no continuous spireme at this stage, but the chromatin is present in the form of a number of segments equal to the spermatocyte number of chromosomes.

At this time begins a change which has rarely been observed in male cells. The chromatin segments begin slowly to break down, and their substance becomes aggregated about the accessory chromosome, thus apparently increasing the size of this body enormously. The disintegration of the chromatin threads is very gradual, as is shown by comparing Figures 11–14. In Figures 12, 13, the process is well begun, and the remaining chromatin segments are very diffuse and flaky, although they still stain like ordinary chromatin, the only difference apparently being that the granules are farther apart. From this fact it is, I believe, impossible to argue that the chromatin is actually dissolved and the solution deposited in or about the accessory chromosome. On the contrary, the mass of granules is merely rearranged and aggregated without change in chemical state about this element. It is merely a mechanical rearrangement of the chromomeres, not a chemical change, which takes place. Some idea of the time required to accomplish the complete rearrangement of the elements of the nucleus may be gathered by comparing the size of the cells in the different stages of disintegration (Figs. 11–15).

As the rearrangement of the chromatin proceeds, the framework of the

nuclear vesicle becomes more and more faint, until it finally stains less strongly than does the cytoplasm immediately surrounding the nucleus (Figs. 128, 130). That it is composed entirely of linin there can be no doubt whatever, its non-chromatic character being shown both by its general appearance and by micro-chemical tests. Here and there in this lightly staining network are suspended coarse, deeply staining granules, which are little larger than centrosomes. These are probably granules of metaplasm deposited in the nucleus as reserve food material. Such granules are also found more or less numerously in the cytoplasm outside the nuclear membrane. They differ from centrosomes in various ways and can be distinguished from them readily. At this period, which, as stated, I shall call the vesicle stage, the chromatin is deposited in a dense mass around the accessory chromosome. This enveloping body, to which I have given the name karyosphere, is situated at one side of the nucleus in close apposition to the nuclear membrane. It is not a single element, but is formed by the aggregation of all the chromosomes about the accessory chromosome as a centre. The karyosphere is not homogeneous, as I believed at the time I wrote my first paper upon Scolopendra. In those studies my observations were made with a magnification of 1000 diameters upon sections $6\frac{2}{3}$ micra thick. In my later work sections 2 to 3 or 4 micra thick were also used, and the objects were observed under a magnification of 1200, 1920,[1] or even 2620 diameters. By means of this increased magnification of very thin sections it was found that the karyosphere is entirely different in structure from what I at first believed. It is a very complex mass of fine chromatin fibres closely enveloping a solid chromatin body. Whether this fibrous mass is composed of the loops of one continuous thread (i. e. is a true spireme), or whether it is in the form of segments, I am unable to state positively. However, I believe the latter to be the case, for reasons which will appear later, when I come to the description of the formation of the chromatin elements from this mass in the ensuing prophase. Often, in the thicker sections in which the process of extraction of the haematoxylin has been carried farther than usual, the karyosphere presents the appearance represented in Figures 19, *b*, 130. In those cases the mass is often bounded

[1] In obtaining a magnification of 1920 diameters, a $\frac{1}{12}$ in. objective with a no. 8 ocular was used, and a beautiful clear image resulted. With a magnification of 2620 diameters the same ocular was used with a $\frac{1}{18}$ in. objective. Here the results were not so satisfactory, and less reliance was placed on the observations. However, the magnification of 1920 diameters was sufficient to show the structure of the element well.

by a definite clear-cut outline, which has almost the appearance of a membrane, although no membrane has been demonstrated, nor, indeed, do I believe one exists. The chromatin is aggregated into several irregular dense masses, and in optical section apparently occupies about three fourths of the area. Surrounding this irregular mass of chromatin is a homogeneous or very finely granular substance. I do not believe that this is a normal condition of the karyosphere, but I think it is the result of changes brought about by the massing together of the chromatin threads under the action of the fixing reagents. The spireme threads, always very close together during this stage, are forced still closer by the shrinking action of the fixative, thus leaving spaces which are occupied only by the karyolymph. In thick sections as usually stained, the minute spaces between the chromatin fibres become filled with the iron-haematoxylin and cannot be decolorized; thus the whole body appears to be homogeneous, as seen in Figure 19, *a*. In the thinner sections and in those which pass through one side of the karyosphere, decolorization proceeds normally, and the result is that the individual chromatin fibres are often visible, as in Figures 19, *c*, *d*, *e*. Sections through one side of the karyosphere are often met with, and are very instructive in showing the true character of this body. Figures 19, *c*, and 22 represent such sections. In the former there is a denser mass of chromatin (probably the accessory chromosome) upon one side, while the remainder of the structure is made up of numerous granular segments in cross section. In Figure 19, *d*, a thin section through the centre of a karyosphere at the same stage, is shown a mass or network of threads, which are at some places closely aggregated, while at others they are more loosely arranged, showing the individual fibres and the karyolymph spaces between. Figure 19, *e*, is a section through a karyosphere in the very early prophase, at about the stage represented in Figures 20, 21. The accessory chromosome is seen at one side, while the fibres have become considerably loosened, showing more plainly the reticular structure of the body.

Thus we see that the karyosphere at this stage contains most of the elements and possesses most of the characteristics of a nucleus. It contains all the chromatin of the cell in the form of a spireme or spiremes. This chromatin is arranged upon a linin framework, as in the ordinary nucleus, and the spaces between the different strands are filled with karyolymph. The only element lacking is the nuclear membrane. Now, it is well known, from the researches of Calkins and numerous other writers upon the cytology of the Protozoa, that many

of the lower types of Protozoa possess nuclei which are only masses of chromatin, or karyosomes, lying free in the cytoplasm without any surrounding membrane whatever. That this is a strictly analogous body in origin, structure, and function there can be no reasonable doubt.

It is evident, then, that morphologically the karyosphere is at this period the real nucleus of the cell. Whether at this time it also functions as a nucleus in metabolism, my observations do not allow me to decide. However, from the structural relations which exist between the karyosphere, the nuclear vesicle, and the other organs of the cell, I do believe we are not justified in drawing such a conclusion. We might rather infer that at this time there is a division of labor, that certain functions which generally pertain to the nucleus as a whole are localized in a highly specialized portion of it, while the rest of the nucleus still retains the powers not so delegated.

We return now to the telophase of the last spermatogonium in order to trace the development of the extra-nuclear structures of the cell. At the time of the constriction of the cell wall the interzonal filaments, drawn out between the separated chromatin plates, are forced together at their equator so as to form a sheaf-like bundle (Figs. 7, 8). At this time, also, the centrosomes are to be seen at the end of the cell farthest from this region of constriction, surrounded by a mass of archoplasm probably formed by the breaking down of the astral rays and mantle fibres (Fig. 7). As the cell advances in development, the interzonal filaments at the ends farthest from the point of constriction lose their fibrillar character and break down into a loose mass of granules. In the region of constriction the peripheral ones thicken into a number of dark bodies, such as are characteristic of the spindle remnants of cells rich in archoplasm, and form a band encircling the remaining filaments. The equatorial part of these remnants of the spindle is granular and continues so as long as any of it persists.

Very soon after the stage represented in Figure 7, each pair of centrosomes with their surrounding archoplasm moves through the cytoplasm around the mass of disintegrating chromosomes and finally comes to rest at the ends of the remnants of the spindle (Figs. 9, 10). Here the archoplasm surrounding the centrosomes becomes closely apposed to the disintegrating archoplasm of the spindle remnants. The two masses do not unite, however, but can be distinguished from each other throughout all the subsequent changes (Figs. 12–16). From this time the centrosome can be traced through the astonishing growth period, the two subsequent divisions, the spermatid, and to its final position in

the mid-piece of the spermazoön. In Figures 7 and 8 is shown the beginning of that enormous growth which is so characteristic of the early spermatocytes of all chilopods.[1]

In a former paper (Blackman, :01) I laid stress upon this unusual growth and called attention to the resemblance between the spermatocyte in the vesicle stage and the female germ cell of a like stage. The resemblance is indeed extraordinary, and extends in many respects even to the most minute particulars. As the growth of the cell continues, the cytosome increases in size much faster than does the nucleus; whereas formerly, in the spermatogonium, the nuclear vesicle enclosed the greater part of the contents of the cell, now it contains only a relatively small portion. As is readily seen (Figs. 10–16), however, it enlarges also. The cytoplasm during its increase in amount also changes considerably in appearance. During the division stages of the spermatogonium it appears more or less granular with very fine fibrils in the region of the centrosomes, while at other places it is finely reticular in structure. As the growth continues, this reticulation becomes more pronounced (Plate 8, Fig. 127), and at the stage of the completed vesicle the network is often so coarse as to give the cytoplasm a ragged appearance (Figs. 128, 130).

As the cell grows, its appearance is also greatly modified by the increase and arrangement of the archoplasm in a layer about the nucleus of the cell (Figs. 15, 16, 128–130). The zones of archoplasm of the two spermatocytes arising from one spermatogonium are furthermore connected by a bridge of fibrous character. This bridge is the last trace of the interzonal filaments of the preceding division, and even in the vesicle stage is often very distinct and well defined (Figs. 16, 128). At the central portion of this mass of persisting spindle remnants the small dark-colored bodies, often designated as the "Zwischenkörperchen," are generally shown distinctly (Figs. 13, 14). These are arranged in a circle around the periphery of the bundle at the plane of constriction. During the telophase of the subsequent division, the corresponding

[1] The author has examined the five principal genera of Chilopoda, and all these are characterized by an immense increase in the size of the spermatocyte over that of the spermatogonium. This is slightly more marked in Lithobius than in Scolopendra, Scutigera, and Geophilus, and is least noticeable in Scolopocryptops. In the latter genus the cells are much smaller than in any of the others, but they are still characterized by the relatively large amount of cytoplasm which is common to all chilopods. It is my purpose to make a comparative study of the spermatocyte changes of these genera of chilopods, and therefore I shall not enter into more detail here.

bodies are united together into a ring-like band, as described by McGregor ('99) for Amphiuma; but during this earlier stage the ring is composed of a number of separate bodies in close apposition to each other. The central portion of the spindle remnants extending through the opening in each of the two cells is still plainly of a fibrous character, although the component parts are often somewhat masked by the reticulations of the less modified archoplasm surrounding it. The remnants of the spindle proper fuse indistinguishably with the mantle of archoplasm surrounding the nucleus. This, as I have already said, is of a reticular structure similar to the cytoplasm, of which it is indeed only a slightly modified form. It differs from this, however, in being more dense, — i. e. the network encloses finer meshes — and in containing at certain regions depositions of fine granules. These thickenings are generally toward the periphery of the archoplasmic zone, and are often so pronounced as to suggest the presence of a definite membrane. In other cells, however, this outer layer is broken up into a number of irregular portions, which plainly consist of a network in the meshes of which are enclosed numerous granules. When examined under low powers, these bodies seem to be homogeneous or of a very finely granular structure, but with higher magnification the true structure is evident. In the inner zone the reticular character of the archoplasm is much more evident, as here the structure is not so obscured by the deposition of granules. This region is but little more dense than the undifferentiated cytoplasm or the network within the nuclear vesicle.

In the outer dense area of archoplasm there is always an irregular ovoid or globular body containing two small darkly stained bodies (Figs. 16, 133). This body, which is the "Idiozome" of Meves, or attraction-sphere, is not identical in structure with the adjacent archoplasm, but is denser, and exhibits no reticulations even with the highest magnification available. In this idiozome there is no differentiation into zones, as has often been described for other objects, but it appears to be a simple granular mass containing two denser bodies. This mass probably represents that part of the archoplasm which is derived from the mantle fibres and astral rays, and in the telophase of the spermatogonium surrounds the centrosomes during their migration around the mass of chromosomes (Figs. 7 and 9). The identity of the idiozome with this body is plainly shown in Figures 7–16.

Besides the centrosomes there is generally a rather large number of coarse granules distributed throughout the archoplasm and the undifferentiated cytoplasm at various points. These, however, differ from the

centrosomes in staining reaction and in general shape and appearance. When stained with iron-haematoxylin alone the only apparent differences are in their irregular shape and indefinite position. But in slides where the decolorizing has been carried farther than usual, these lose their black stain, while the centrosomes retain their color much longer. Then, too, in the double stain with Heidenhain's iron-haematoxylin and Congo red the centrosomes stain an intense black, while the cytoplasmic granules are of a brownish red color.

As to the nature and function of the granules I can say nothing definite, but I consider it probable that they are metaplasm. This term as generally used may, I believe, be applied to any lifeless substance found in the protoplasm of the cell, and has often been used to designate both reserve food-material and by-products or secretions of the cell which no longer function in the cell's activity. That such is the nature of these granules is indicated very strongly, although of course not proved, by the fact that, during the active stages of the following mitoses, when all the other constituents of the cell change in a greater or less degree, these bodies remain passive in the cytoplasm and seem to undergo no change whatever. This view is further strengthened by the fact that at various places in the interstices between the membranes of adjacent cells there are numerous aggregations of a substance similar in general appearance and in staining reactions to these metaplasmic bodies. Their presence outside the membrane offers no evidence as to whether they are excretions or food material. It is probable, however, that both materials are here represented, since there seems to be some diversity of staining reaction and they vary much in size and general appearance. The larger ones have much the appearance of oil droplets. They possess clear-cut outlines and stain in the characteristic manner with osmic acid, while the smaller ones are more irregular in outline and stain differently. The best proof of the oleaginous character of the larger ones is found in the material fixed in Flemming's fluid. Here these bodies exhibit the reaction so characteristic of fat droplets when treated with osmic acid.

The cytoplasm near the cell membrane is considerably denser than that occupying the rest of the cell. It seems here to form a layer, the inner part of which is only a little more closely woven than is generally the case in these cells. In this layer the cytoplasm, as one proceeds outward, becomes more and more dense until at the periphery it forms a structure which serves as the limiting membrane of the cell. It is evident, I think, that in Scolopendra the cell membrane is but a slightly modified thickening of the ordinary cytoplasm. Indeed this structure is

not at all well developed, and often when seen in very thin sections plainly shows its reticular character. It is much less clearly marked than the nuclear membrane, for while the latter stands out very plainly in the vesicle stage, the former is often very difficult to distinguish as a definite structure.

The spermatocytes when they have reached the vesicle stage are of two well-defined types. The history of one of these types, the smaller, was described in considerable detail in my first paper (Blackman, :01) on the spermatogenesis of Scolopendra. The larger type was merely mentioned there, but a second paper (Blackman, :03) deals with these larger ones almost exclusively. As the succeeding changes in the cells of the two types present considerable differences, I think it advisable to characterize them briefly at this time. The differences between the two types of cells are not at all conspicuous in the vesicle stage, and were it not for the more striking discrepancies which appear later, would not warrant a separate treatment of the two. We have seen that during the growth period some of the cells remain connected to each other by the persistence of the spindle remnants, even up to the time when the vesicle is fully formed. The cells which remain thus united in pairs develop into the large type of spermatocyte. They are always drawn out in the form of two cones with bases in contact and apices directed toward the opposite ends of the follicle, — i. e. they are arranged with their longest axes parallel to that of the follicle. The cell membrane separating the two individuals of a pair is not nearly so well developed as that surrounding the rest of the cell, and it shows more plainly its reticular character. It appears to be only a very slightly modified and condensed form of cytoplasm. Furthermore, as I have said, there is a well-defined opening in this part of the cell membrane through which the interzonal filaments extend. This sheaf of persisting filaments is continuous in each cell with the mantle of archoplasm surrounding the nucleus and is itself surrounded by masses of archoplasm, the characteristic fibrillar structure often being so masked as to be apparently obliterated. The archoplasm has the form of a mantle completely enveloping the nucleus, and is differentiated into two fairly well-defined layers, an outer, more dense layer and an inner one, which differs only a little from the ordinary cytoplasm. Both of these layers are reticular and enclose in their meshes accumulations of very fine granules, which are more noticeable in the outer than in the inner portion. In addition to these archoplasmic masses there is another accumulation of a purely granular nature, which encloses the two deeply staining centrosomes.

The cytosome of the large type of spermatocytes is of relatively enormous size (60 to 90 micra in diameter), and exhibits in a beautiful manner the characteristic structure of the cytoplasm. The nucleus, which at this stage is surrounded by a delicate but well-defined membrane, is also filled with a regular network of linin fibres, in the meshes and at the nodal points of which are here and there imbedded coarse granules of metaplasm. The entire chromatin of the cell has the form of a spireme or spiremes closely packed about the accessory chromosome (Fig. 19, *a–e*). This mass of chromatin is the karyosphere.

The differences from these conditions presented by the small type are, exclusive of size, few and apparently unimportant, but that they are of some significance is demonstrated by comparing the later behavior of the two kinds of cells. They may be briefly summarized as follows: During the growth period, at the time of the rearrangement of the chromatin segments into the karyosphere, the two cells derived from a spermatogonium become separated in such a way that the intercellular bridge formerly connecting them is destroyed, the protoplasm of the two cells being no longer continuous. This is perhaps correlated with the fact that, although the two cells remain very close together, and, indeed, are often in contact, development does not continue at exactly the same rate in both. The result is that the two daughter cells from one spermatogonium, instead of being of the same size and in exactly the same stage of development, present differences which, though usually slight, are often more marked. Frequently cells, though in the same stage of mitosis, show such differences in size as are seen in Figure 57 (Plate 5). On the other hand, two sister cells may be in quite different stages of development (Fig. 156). However, the most striking variations in the vesicle stage are due to the much smaller size of one type and the disproportions in the size of the different parts of the cell. As I have elsewhere stated, the spermatocyte of the smaller type varies considerably in volume, but the diameter of the average cell is about 35 micra, whereas that of the larger type is about 70 micra. In the smaller spermatocytes the nucleus is considerably larger in proportion to the amount of cytoplasm than in the larger type, although otherwise similar to it in all essentials. The smaller size of the cytosome seems to result in a smaller amount of archoplasm. The amount of archoplasm may also be influenced possibly by the absence of a connection between the two cells of a pair.

Another difference exhibited by the two types of cells concerns their position in the testis. As I have pointed out, the cells of the larger

type are always arranged in pairs, with their long axes parallel to the longest diameter of the follicle. This is not true of the smaller type. Possibly owing to some inequality of pressure, the cells of a pair in the testis have apparently been forced apart or caused to rotate upon each other in such a way that the bridge connecting them has been destroyed and their symmetrical arrangement within the follicle has been disturbed. At any rate, they no longer preserve the ideal arrangement, but their longer axes may form any angle with that of the testis. The consequence is that, while the cells of the large type are arranged loosely in the testis and are surrounded by the reticular matrix which I have described as existing in the intercellular spaces, those of the smaller type are packed closely, so that the interspaces between the cells are more nearly obliterated; indeed, the cells of this type lose their spheroidal form, and by pressure are converted into irregular polyhedrons. Thus the mechanical conditions affecting the cells of the large type are quite similar to those of the typical growing ovum, whereas the environment of the smaller type is more nearly that of the ordinary testicular cell. The two types of cells resemble respectively the oöcyte and the ordinary spermatocyte. This fact, and the conditions observed in later stages, allow us to affirm that cells of the smaller type differ from the ordinary male sex cell much less than do those of the larger type, which show in general and in many details the changes which are usually considered characteristic of egg cells.

### 3. Abnormal Cells.

Besides these two well-established types, there are occasionally seen in the testis abnormal variations of the cells during the vesicle stage. Such a one is represented in Figure 139. These cells differ considerably from the normal cells of like stage. The total amount of chromatin to each cell is many times that ordinarily present in the vesicle stage. This chromatin is in the form of from one to ten, or even twelve, apparently homogeneous spherical masses connected by an extremely coarse deeply staining system of chromatin bands arranged in the form of a network.

The cytoplasmic structures also present marked differences. The archoplasm is apparently scanty, and is scattered in small fragments here and there throughout the cytoplasm. No centrosomes are visible, even after the most careful search. The cytoplasm is much coarser and

denser than ordinarily, and the meshes are filled with numerous granules and globules of an oil-like substance.

Cells showing these abnormal conditions arise at the extreme periphery of the follicles only, and are usually separated from normal cells of the same stage by numerous cells of a younger generation. The explanation of these abnormal cases is, I believe, obvious. By the too rapid propagation of the adjacent cells an inequality of pressure has ensued, which has caused these cells to be forced to the periphery of the testis instead of toward the centre, as ordinarily occurs. Here they are nearer the base of food supply, and the consequence is a pathological development, resulting in the hypertrophy of the chromatin and the distortion and obscuring of the cytoplasmic structures.

That these cells are abnormal or pathological is shown by their later changes. They never accomplish the spermatocyte divisions, but eventually degenerate. Such cells in diverse stages of degeneration may be occasionally seen at various places in the testis and vas deferens. They are eventually forced from their peripheral position by the pressure incident to the continued propagation of the adjacent spermatogonia. As soon as they leave this position they always begin to show signs of degeneration, if, indeed, such have not become evident before. The chromatin becomes granular and spongy, and no longer reacts to the stains in a normal manner. As this retrogressive process continues, the cytoplasm decreases more and more and becomes loaded with the degenerating chromatin derived from the nucleus. This continues until a division between nucleus and cytoplasm seems no longer to exist, and the whole cell, much decreased in size, is filled with a dense mass of granules which stain brown.

The decrease in the size of the cell during these stages suggests that it may possibly serve as food for the normal cells. Although this is probably the case, I do not believe that certain cells are normally set apart for this purpose.

In following the later changes in the spermatocytes of Scolopendra, it will prevent confusion if the two types are taken up and considered in detail separately. In doing this I shall first give the history of the changes in the large type, and next shall consider the small type, calling attention to the points of similarity and difference, and attempting an explanation of these differences.

## 4. Maturation Divisions in the Large Spermatocytes.

### A. *Division of the First Spermatocytes.*

In the prophase of the spermatocytes of the large type, the first changes observable have to do with the centrosomes and archoplasm. The two centrosomes, which up to this time have apparently lain passive in the cytoplasm surrounded by their special envelope of archoplasm, now show signs of activity. The two parts, heretofore quite distinct, come together and partly coalesce to form a dumbbell figure which, surrounded by a small sphere of archoplasm, then begins to migrate toward the nucleus. During this migration the idiozome gradually diminishes in size, and synchronously very faint astral rays begin to appear. Finally by the time the centrosomes reach the nuclear membrane the granular structure of the centrosphere has disappeared, and this body is now represented by a small colorless, structureless mass surrounding the centrosomes, through which the feebly developed astral rays extend (Fig. 20). The centrosphere at this time fails to take the stain, and is apparently composed of the same substance as that occupying the interstices of the cytoplasmic reticulum.

While these changes are occurring, the archoplasm surrounding the entire nucleus disintegrates and upon superficial examination seems to have entirely disappeared. The cytoplasm has also altered considerably in appearance. Its reticular structure is no longer as apparent as formerly, and it retains the stain more strongly and appears considerably more dense. The archoplasm as it disintegrates is converted into a liquid or semi-liquid very finely granular mass, which is disposed evenly throughout the cell in the meshes of the cytoplasm. Here, mixing with the fluid hyaloplasm, it causes this inter-reticular substance to stain much more deeply, and thus alters considerably the general appearance of the cytosome.

As the compound centrosome approaches the nucleus, the nuclear membrane is often drawn out into a cone-shaped protuberance, as shown in Figure 20. This is probably caused by an attractive force exerted by the centrosome. Soon after reaching the membrane the centrosome again divides into its two parts, which separate and begin their slow migration toward opposite poles of the nucleus.

In his work upon the spermatocytes of Scolopendra dalmatica Carnoy ('85), however, states that the centrosomes do not migrate apart upon the nuclear membrane, but that they move through the cytoplasm at a

distance from that structure, and having described an arc of 180°, take up their position in the cytoplasm between the nucleus and the cell membrane. Other investigators upon chilopods — Meves und von Korff (:01) for Lithobius forficatus; P. Bouin (:01) for Lithobius forficatus; and P. Bouin et R. Collin (:01) for Geophilus linearis — have arrived at similar results. That this never occurs in the first spermatocyte division of Scolopendra heros, I can state with certainty after having observed several hundred cells in the prophase and in other stages of mitosis. There can be no doubt, however, that such phenomena do occur in other genera of Chilopoda (Lithobius, Scutigera, Geophilus), where I have myself observed them. In the second spermatocyte of Scolopendra there is present a condition similar in some respects to that described by Carnoy, and it is possible that these cells have been mistaken by him for first spermatocytes.

As the centrosomes continue to diverge upon the nuclear membrane, the astral rays surrounding them become more and more marked. Whereas at first these were of a granular nature, now they become more clear cut and approach more nearly the fibrillar structure characteristic of them during the succeeding metaphase. Before the centrosomes have separated very far, each one becomes elongated in a plane tangent to the nuclear membrane and exhibits signs of a second division (Figs. 21, 22). This division, however, is not fully accomplished until the following metaphase. Often during this period of the prophase the nuclear membrane is distorted in the vicinity of the centrosomes, giving it the appearance of undergoing amoeboid movement. In some cases this seems to be due to an attractive influence exerted by the centrosomes, as is shown by the existence of protuberances (Fig. 22), but in other cases the membrane is affected in exactly the opposite way (Fig. 21), which suggests a repellent force on the part of the centrosome. This distortion of the nuclear vesicle is most marked at the stage immediately preceding the disintegration of its membrane (Figs. 29, 30). At this time, when the two compound centrosomes are at opposite poles of the nucleus, the diameter of the vesicle connecting the two centrosomes is considerably shorter than those at right angles to it (Figs. 29, 30).

At this stage the centrosomes are never immediately in contact with the membrane, but are at a distance from it equal to perhaps half of their own diameter. That part of the nuclear membrane which is near the centrosome is invariably bent inward, forming a cup-like depression, in which the double centrosome lies. Under high magnification it is

seen that the nuclear wall forming this depression is in process of disintegration, and the linin of the nuclear space is becoming arranged into fibres which point toward the centrosome and are apparently continuous with the short astral rays upon the side of the centrosome nearest the nucleus (Plate 4, Figs. 48, 49).

While these phenomena have been taking place in the cytoplasmic structures of the cell, the nucleus has also undergone some very marked changes. At about the time the centrosomes begin their divergent courses upon the nuclear membrane the appearance of the karyosphere is considerably modified. It no longer preserves the sharp outline which, as a general thing, is characteristic of it during the vesicle stage; but even in thick, darkly stained sections its contour appears irregular and its periphery seems granular. Upon studying thin sections this change in appearance is explained. The dense mass of chromatin threads forming the karyosphere is becoming more loosely arranged. The spireme is no longer so closely packed as formerly, but in favorable sections appears as shown in Figure 19, *e*, or as in its tangential section seen in Figure 22. Figure 19, *e* is a section through the centre of the karyosphere, and shows upon one side a dense body (doubtless the accessory chromosome), while the remainder of the chromatin is in the form of a loose spireme. In Figure 22 the karyosphere is cut through one side, showing at certain places denser aggregations of the chromatin granules. These aggregations undoubtedly represent cross sections of chromatin threads.

This loosening of the spireme becomes more and more marked, until the regular contour of the karyosphere is entirely lost and chromatin threads project at several points upon its surface. These projecting ends rapidly lengthen by the unwinding of the chromatin filaments, and when they have attained a certain length, become detached, forming slender segments, which are equal in number to the number of chromosomes in the succeeding metaphase. As the number of these chromatin segments present in the nuclear vesicle increases, the size of the karyosphere is proportionately decreased, so that it would seem to follow that the chromosomes are derived from this peculiar body. Even if it were not possible to give more definite evidence from actual observations of all the stages incident to such an origin of the chromosomes, this would probably be a just assumption, although there would still be room for doubt. But as these stages exist and are very numerous in my material, I think it is impossible for the observer to escape the conclusions I have drawn from them.

Various stages in the disintegration of the karyosphere and the formation of the tetrads are shown in Figures 21–28 (Plate **2**) and 136, 138, 141–144 (Plate **8**). Figure 20 shows this body at the beginning of the prophase before the appearance of the chromosomes; it is from a thin section, and shows excellently the true reticular nature of the karyosphere. In Figures 23, 24, are shown camera-lucida drawings which illustrate the origin of the chromosomes as well as could be done by means of diagrams. In Figure 24 the karyosphere, much reduced in size and apparently not homogeneous, has given rise to three granular thread-like processes. These processes are similar in all respects to the typical chromatin filament in the spireme stages. Near the distal end of each of these threads there is a filament of chromatin which apparently has just become detached from the mass and has already segmented longitudinally in the manner characteristic of chromatin segments in the prophase. Practically the same stages are shown in Figures 143, 144, and in Figures 23, 25, 27. In Figures 25, 28, 142, is shown a considerably later stage, wherein the last chromosomes to be formed are seen arising from the karyosphere and the accessory chromosome, which still retains its characteristic definite outline and homogeneous structure, is again plainly distinguishable. When these conditions, shown both in camera-lucida drawings and in photo-micrographs, are considered, I believe no one will hesitate to agree with the conclusion that during the vesicle stage all the chromatin of the cell is contained in the karyosphere and that during the subsequent prophase this body gives rise to the chromosomes.

During the vesicle stage the karyosome consists of a number of very attenuated chromatin segments closely massed about the accessory chromosome. In the early prophase following, the first change noticeable is the loosening of this mass of threads (Fig. 20). Later, several ends of this filamentous sphere become free, and by the simple process of uncoiling give rise to long granular processes extending out into the linin network (Figs. 23, 24). These protruded threads detach themselves, and new projections appear in their place until sixteen segments are present (Fig. 28). This is the number of segments present in the early spermatocyte — i. e. before the vesicle stage — and the number of chromosomes later seen in the metaphase, exclusive of the accessory chromosome. When all of these segments have arisen from the karyosphere, nothing remains but the accessory chromosome, which has formed the core of this structure (Figs. 25, 28, 142).

As the chromatin segments arise from the karyosphere, they are long

granular threads, often considerably curved or distorted in various ways. They rapidly become straighter and soon show signs of longitudinal cleavage. In Figure 24 most of the chromatin segments are in this condition, i. e. they consist of more or less curved granular rods each of which has been longitudinally split into two parts. Since it is to the prophase that we should look for indications as to whether the first spermatocyte division is one of equation or reduction, it is of importance to note here that the first indications in the prophase point to a *longitudinal* division. As the longitudinal division of the chromatin segments is the first which occurs in the prophase, I think it very probable that it is the one completed by the first maturation mitosis.

What is apparently the next change in the appearance of the split segments is shown in Figures 26, 27. This first becomes evident as a "weakening" of the two parts of the segment at about their middle. The threads show a tendency to bend at a more or less sharp angle at this point (Fig. 26, A), and this soon results in a transverse division of each of the parts of the chromosome. Thus, each of the chromatin segments has been divided into four parts and may from now on be called a tetrad. Following the terminology suggested by McClung, I shall designate each of the parts going to make up the tetrad or chromosome of the first spermatocyte, a chromatid. By this I believe confusion will be prevented.

After transverse division has become established, the next change observable is shown in Figure A, *a*, *b*, *c*. The chromatids revolve upon each other in such a manner that the ends at the point of transverse cleavage are drawn out parallel to each other, and an irregular cross-shaped figure is thus formed (Fig. A, *d*, *e*). This cross-shaped figure is composed of four arms of about equal length, each of which is split longitudinally. Owing to the very irregular shape of these arms, the cleavages are masked and are often very hard to demonstrate. However, in later stages, when the arms are greatly shortened, the bipartite structure is readily seen (Fig. B, *a*). It is also strongly indicated, even in the earlier stage, by the diamond-shaped opening at the centre of the tetrads. When viewed *en face* this opening is always square or diamond-shaped, with the angles directed toward the arms, indicating that the opening is continuous into the arm.

At the stage represented in Figure A, *e*, *f*, the tetrads are often so distorted that the typical form is lost, but upon studying them more carefully it is seen that they are always referable to the same type. Taking *d* as the type, the more common variations are shown in *b*, *c*, *e*,

*f*, *g*, and *h*. At *b*, the formation of the arms instead of occurring in the plane of the threads has proceeded in a plane at right angles to it, resulting in the double-V figures first mentioned by Paulmier ('98). At *c*, *h*, the long arms of the cross have been curved around and nearly brought into contact. Such distortions observed in later stages of tetrads result in a figure similar in shape to a seal ring, the point of double cleavage representing the seal and the long arms meeting to form an apparently closed circle. Figure A, *e*, *f*, *g*, are but slight or apparent modifications caused by viewing the tetrads diagonally or in profile.

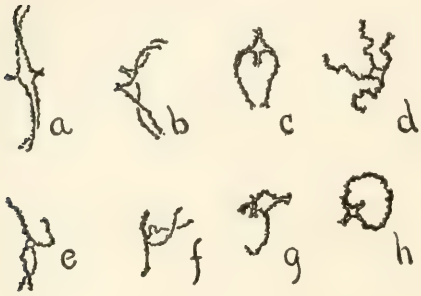


FIG. A.

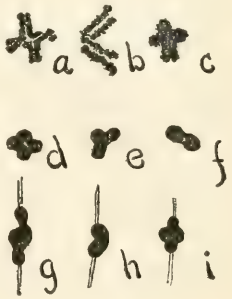


FIG. B.

FIG. A. Various stages and modifications of tetrads: *a*, *b*, *c*, early stages in the process of transverse division; *d*, typical tetrad of mid-prophase; *e*, *f*, *g*, *h*, modifications of the tetrad type. × 1,400 dia.

FIG. B. Later stages in the history of the tetrad: *a*, typical cruciform tetrad of late prophase; *b*, "double-V" form of chromosome at the same stage; *c*, *d*, successively later stages of the cross figure; *e*, *f*, apparent modifications of tetrad in later prophase; *h*, *i*, typical chromosomes at beginning of metaphase; *g*, tetrad undergoing longitudinal division. × 1,440 dia.

By later changes the arms of the cross-like figures are much shortened and the divisions of the separate chromatids become very apparent (Fig. B, *a*, *b*, *c*). However, this shortening and condensation continuing, these divisions are entirely obliterated, and the chromosome becomes first a granular mass and later apparently homogeneous. The chromosomes even at this stage vary considerably in shape, as is shown in Figure B, *d*, *e*, *f*, and Figure 29. The typical form is represented by *d* (Fig. A) and by numerous chromosomes in Figures 28, 29, 30. During the prophase the tetrads of the same nucleus have not developed at the same rate, but, at any given time, the chromosomes of the same cell are in various stages of transformation. When the origin of these elements is

considered, this behavior is naturally expected. Owing to the compact manner in which the extremely fine chromatin threads are aggregated in the karyosphere, only a few chromosomes can arise from it at one time, so that naturally those which are first formed are in more advanced stages of development than those that appear later (Fig. 25). However, by the time the centrosomes are upon opposite sides of the nucleus and the nuclear membrane has begun to disintegrate, all of the chromosomes are in apparently the same stages (Figs. 29, 30). All are dense, homogeneous structures, which take the stain with avidity and retain it strongly.

While the other chromosomes have been undergoing these changes in structure, the accessory chromosome has also changed in shape. It has apparently at no time lost its homogeneous structure, but that it has undergone important changes is evident. It is no longer a spherical body, but now has the form of a rod, the ends of which are slightly notched (Figs. 29, 30, Fig. C). These notches I believe indicate a longitudinal division. The accessory chromosome differs from the other chromosomes in shape and is evidently not of a tetrad nature. When its origin is considered this difference in form is very easily explained, and is indeed exactly what would be expected. The other chromosomes are formed by a fusion of two of the spermatogonial chromosomes into one body during the telophase of the last mitosis of the division period. On the other hand the modified accessory chromosome is obtained directly from a single chromosome of the spermatogonium. This being true, it would be but logical to expect it to act differently. The primary object in the division of the spermatocyte is the reduction of the chromosomes to one half the somatic number. This is generally if not invariably accom-

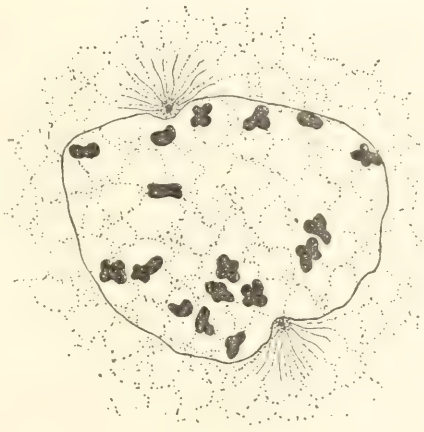

FIG. C.

FIG. C. Nucleus of first spermatocyte during late prophase, showing various modifications in the shape of the chromosomes at this time. The accessory chromosome is seen to be notched at the ends. Centrosomes with well-developed astral rays at opposite poles of the nucleus. × 1,440 dia.

plished, in arthropods at least, by one cross and one longitudinal division. It is generally assumed that, by one of these divisions, — the transverse division, — this reduction is accomplished through the breaking apart of the chromosomes at the point at which they were united in the preceding synapsis. Now, as the accessory chromosome is not obtained by the union of two spermatogonial chromosomes, this reduction division is not necessary for it and does not take place. For these reasons, while the ordinary chromosomes are each composed of four parts, i. e. are tetrads, this modified chromosome is made up of but two parts, i. e. is a dyad. Furthermore, it is logically to be expected that the accessory chromosome, being dyad in its nature, should take part in only one of the succeeding divisions. This peculiarity has indeed been observed by many investigators of insect spermatogenesis, and several hypotheses more or less supported by observed facts are offered in explanation of it. These theories I shall mention later, in my discussion of the pertinent literature.

While these important changes have been taking place in the centrosome and in the nuclear structures, the cytoplasm has remained in practically the same condition in which we left it in the very early prophase. It is reticular in structure, but the network is obscured by the fact that the interstitial substance no longer remains uncolored, but retains the stain with considerable strength. This increased staining power of the hyaloplasm I believe to be caused by the dissolving of the archoplasm and its mixing with the liquid or viscid substance which fills all the inter-reticular spaces.

When the cell has reached the stage shown in Figures 29 and 30, the nuclear membrane begins to disintegrate. This is first apparent in the parts nearest the centrosomes. The membrane apparently becomes granular, and the fibres of the nuclear network begin to arrange themselves in lines which converge toward the centrosome (Figs. 29, 30). The astral rays developed outside the nucleus now seem to penetrate into the nuclear space (Fig. 31). In some cases these rays seem to unite with those formed by the linin fibres, while in others the two sets seem to remain distinct. I think it very probable from these observations that the linin reticulum gives rise to the mantle fibres, while the astral rays upon the side of the centrosome nearest the nucleus form those fibres of the spindle which do not connect with the chromosomes, i. e. the spindle fibres. As the nuclear membrane continues to disintegrate, the centrosomes move apart and thus draw the converging linin threads out into a spindle-shaped figure (Fig. 31). By this same movement the chromosomes are also drawn into a position between the centro-

somes, where they are arranged in a very irregular manner (Figs. 31, 32). There is no true equational plate established, for the chromosomes are not arranged in one plane at any period of mitosis.

The spindle as established in the large type of spermatocyte has a very constant and definite relation to the long axis of the cell and of the testis. In all cases observed, it is perpendicular to the long diameter of the cell, and as the cells are always arranged parallel to the follicle, the spindle as first constructed is perpendicular to the long axis of the testis. The constancy with which this arrangement of the spindle occurs at this stage suggests that the cell may have a distinct polarity in the early stages as well. But this is not entirely the case. While there is a very definite relation between the Zwischenkörper and the nuclei of the two connected cells, this does not extend to the other organs of the cell. The centrosome with its surrounding substance may apparently be situated at any point within the outer zone of archoplasm. However, during the early prophase it often so arranges itself upon the side of the nucleus opposite the persisting spindle, that a plane passed through these two structures divides the nucleus and the cell body into two approximately equal parts (Figs. 16, 17). Even at this stage, however, the karyosphere is not involved in this polarity of the cell contents, but may apparently occupy any position relative to the plane of division.

As the spindle is formed, several interesting changes in the cytosome take place. The cell outline formerly cone-shaped rounds off until it is in the form of a more or less regular oval (Figs. 30, 31, 32). The cytoplasm also undergoes interesting changes. The inter-reticular substance in the proximity of the nucleus loses the dense appearance which it had assumed during the prophase and again takes on its characteristic structure. As the subsequent stages of mitosis proceed, this return of the cytoplasm to its normal reticular condition continues, until finally, in the late anaphase, it has entirely resumed its habitual character. These changes are due to the loss on the part of the cytoplasm of that staining capacity characteristic of it during the prophase. It is very important to note that while the cytoplasm is assuming its wonted appearance fundamental changes are apparent in other elements of the cell. The astral radiations, which during the prophase are not exceptionally well marked, increase rapidly during the metaphase and anaphase, until at the conclusion of the latter stage they apparently permeate every part of the cell. This growth of the astral systems does not occur at the expense of the cytoplasmic reticulum, for, while this network is indeed somewhat obscured by the astral fibres, it is still present and of

the same character as at other times. The most reasonable explanation of the cytoplasmic changes is the one I have already suggested. In the early prophase the archoplasm is dissolved in the hyaloplasm, and in this condition is present in all parts of the cytosome. During the rest of the prophase it remains in this condition, the only evidence of its presence being the deeper stain which the inter-reticular substance at this time assumes. However, at the time of the establishment of the division figure this substance again appears, now in its kinetic, fibrillar form, and continues to become more marked, until, in the period of greatest cellular activity, during the anaphase and telophase, every part of the cell is penetrated by these archoplasmic fibres. In the metaphase and succeeding stages these threads are much more numerous in the region of the centrosomes than elsewhere in the cell (Figs. 32 *et seq.*). Many of them at these points are apparently short, extending only a little distance into the cytoplasm. Indeed at this period these small fibres form the outer zone of the centrosphere (Plate **4**, Figs. 49, 50).

Of the behavior of the centrosome during these stages I will speak in detail later, but would like to describe at this time its general appearance. During the metaphase and early anaphase the centrosome is a bipartite body of an irregular dumbbell shape contained in, or surrounded by, a centrosphere composed of two layers or zones (Figs. 50, 51). The inner of these zones stains in a fairly definite manner, but much less deeply than the centrosome itself. It is of a homogeneous or very finely granular consistency and of an oval, or later of a lobate form. The outer zone, as I have said, is formed by the closely apposed bases of the astral rays, and has no distinct outline. In preparations stained in the ordinary manner with iron-haematoxylin, it is darker in color than the inner zone, but upon further decolorizing, this dark stain is lost and it appears lighter. In the deeply stained preparations the spaces between the fibres are filled with masses of the stain, but in the lighter-colored sections these deposits are removed and the fibrillar nature of this zone is disclosed.

The longitudinal axis of centrosomes and centrosphere bears no constant relation to the direction of the spindle during the metaphase and anaphase, but during the telophase is always parallel to the plane of cell cleavage.

The spindle as first formed is of symmetrical shape (Plate **8**, Fig. 145), but very soon changes begin which destroy this symmetry (Plate **3**, Fig. 33). When it first appears it extends at right angles to the length of the cell, but the poles of the spindle soon show a tendency to move

toward opposite ends of the cell. This results in a distortion of the spindle, as shown in Figures 33–35, 147. This distortion is really the expression of a rotation of the mitotic figure, such as often occurs in the maturation mitosis of eggs. In this rotation the centrosomes always lead, and very plainly seem to exert an attractive force upon the remainder of the spindle. In Figure 145 is shown a metaphase in which the formation of the spindle has just been completed and the rotation has not yet begun. Figure 33 shows the spindle at the time these distortions first begin, and the same is shown in the fragment of the spindle in Figure 147. Figures 34, 35, represent later stages, in which the revolution is nearly accomplished. As will be seen from the accompanying drawings, this change in the position of the mitotic figure occurs during the metaphase — i. e. before the chromosomes have been divided and have begun to move toward the poles. However, as soon as the spindle is oriented lengthwise of the cell the separation of the chromosomes begins and the cell passes into the anaphase (Fig. 35).

The rotation of the spindle in this manner accomplishes two things which are beneficial to the cell. First, the plane of division is so altered that the ensuing cleavage of the cytosome may be accomplished with the least possible expenditure of energy, and by this rotation the centrosomes are brought into closer relation with all parts of the cytoplasm. This apparently aids in the reconstruction of the latent archoplasm, laid down in the hyaloplasmic areas, into its kinetic form, the fibrillar astral rays. By the time the revolution of the spindle is accomplished practically all the archoplasm has been converted into the fibrillar form and the cell is ready for division. It is now very evident that the cytoplasm has a reticular structure, as, indeed, it has had at all times, although during the earlier stages this structure was not easily distinguished owing to the extraordinary opacity of the inter-reticular substance.

When the revolution of the spindle and the reconstruction of the archoplasm has been accomplished, the cell proceeds to divide. The spindle lengthens, and by this elongation the chromosomes are divided and drawn apart into two groups (Fig. 36). The centrosomes move toward the cell membrane (Fig. 36), and finally with the enveloping centrospheres come to rest near its inner surface (Fig. 37). In the early telophase the chromosomes become aggregated into two masses at opposite ends of the cell at a short distance from the centrosomes. They are so closely crowded together that their individuality is apparently lost, and the mass thus formed is contained in a clear vacuole of hyaloplasm (Fig. 38). In the later stages of the telophase the chromosomes separate and become dis-

tributed throughout the vacuole (Fig. 39). They lose their homogeneous appearance and sharp outlines and assume a granular condition. When the constriction of the cell is completed, the nuclear membrane has also reappeared (Fig. 39).

Even during the late anaphase the cell membrane in the region of the equator of the cell has become depressed, thus indicating the beginning of the division of the cytosome. This depression continues to deepen (Fig. 37), until it becomes very evident that it is in reality an annular constriction which is slowly dividing the cell into two approximately equal parts (Fig. 38). The division of the cell body is very different from that usually occurring in the male cells and in all well-differentiated cells, but is in many respects identical with that occurring in the cleavage of the ovum. It is very evident that the division is not accomplished by the mere formation of a single intervening wall between the cells, as is said to be the case in vegetable cells and in some animal cells, for at all stages two membranes can be readily distinguished (Figs. 38, 39), and at the bottom of the constriction it is plainly seen that these two walls are continuous, — i. e. are formed by the mere forcing in of the cell membrane (Fig. 38). During the progress of this division the reticular matrix of the testis which surrounds the spermatocytes of the large type in a thick layer presses into the constriction and completely fills the cavity thus produced (Fig. 38).

During the anaphase the archoplasmic structures of the cell are more marked than at any other time. The astral rays are very numerous and seem to penetrate to every part of the cell. But those which extend toward the equator are both more numerous and more conspicuous than those of any other region (Figs. 35, 36, 37). In many cells they seem to connect the centrosomes with the cell wall in the region of the equator of the cell. When we consider the behavior of the same structure in the other type of spermatocyte, this view would appear to be justified. However this may be, during the subsequent formation of the Zwischenkörper, the astral rays still persist as such, still apparently attach to the constricting cell membrane, and most certainly take no part in the formation of the persisting spindle.

During the telophase the interzonal filaments are collected together into a bundle by the constricting membrane and form a very well-developed Zwischenkörperchen. At first the fibres composing this are quite long and extend from the plane of division about half the distance to the nuclei. Later, however, when the row of central granules appears, they decrease in size. These central granules are at first small bodies

staining black arranged in a circle about the periphery of the bundle (Fig. 38). Soon, however, they fuse together and form a ring (Fig. 39) similar to those found in amphibian material. The spindle remnants of the first spermatocyte in no case persist very long, but gradually disintegrate and eventually disappear entirely. Very often the destruction of this structure is hastened by the rotation of the cells upon each other which seems always to occur either during the late telophase of the first spermatocyte or during the early stages of the second spermatocyte.

During the late telophase the astral systems undergo important changes. Up to the time when the cell membrane is entirely constricted, they decrease in extent but little, if indeed at all. But as soon as this phenomenon is completed, they degenerate rapidly (Fig. 39). However, they do not wholly disappear. Some of the archoplasm persists in the form of fibres radiating from the centrosphere, while the rest is deposited between the meshes of the cytoplasm. It, however, does not long remain inert, for the early prophase of the second spermatocyte is of very short duration, the second spermatocyte division following very closely upon that of the first.

### Centrosome and Centrosphere of First Spermatocyte.

I have said that during the late prophase of the first spermatocyte the centrosomes, while still moving apart along the nuclear membrane, are evidently elongated in a direction parallel to this structure. This elongation is often very pronounced and may even result in the formation of dumbbell forms such as are shown in Figures 21, 22, 47. The substance of the centrosome becomes aggregated at the two ends of the mass into two more or less equal lobes. This is evidently the first sign of the division of the centrosome, which usually is completed in the following metaphase or anaphase. It should be particularly noted at this point that the body in question is the smallest analyzable portion of the structure at the centre of the asters in the prophase, and is the part toward which the radiations extend and in which they end, i. e. it is the centrosome as defined by Boveri.

At this stage (Plate **4**, Fig. 47) there is no well-defined structure enveloping the centrosome, but in a small space around this minute body the cytoplasmic reticulum is absent, thus leaving a clear zone of hyaloplasm (or latent archoplasm) which is traversed by the astral rays only. Later, in the prophase and in the metaphase, however, a distinct centrosphere is present (Figs. 48, 49, 50). All the observations upon the genesis of this structure would seem to justify the conclusion that the centrosphere

is not derived from the body of the centrosome by a simple process of transformation, as has been maintained by several authors, but rather, that it is caused by a formative power exerted by the centrosome over the latent archoplasm contained in the cytolymph. That is, its origin is similar to that of the astral rays, toward the formation of which it later often contributes some of its own material. It is to be especially noted that during all the early stages of the development of the centrosphere, some of the astral rays may be seen to traverse the centrosphere and to abut upon the smaller enclosed body, the centrosome. The same phenomenon is to be observed occasionally in the later metaphase and anaphase. These facts seem to preclude the possibility of the outer zone being considered the true centrosome, and to justify the application of that term to the small dark staining body within. Shortly after the formation of the first maturation spindle the structure at the centre of the aster seems to have reached its highest development (Figs. 50 *a*, 50 *b*). It consists of an elongated dumbbell-shaped centrosome enclosed in a homogeneous or very finely granular centrosphere of an oval form.

In the metaphase of the large type of spermatocyte the long diameter of this oval mass is arranged in no fixed relation to the diameter of the spindle, although later, in the telophase, a constant relation is very apparent (Plate **3**, Figs. 37–39). It is to be noted, however, that during the rotation of the division figure, which in the large type of spermatocyte seems invariably to occur during this stage (metaphase), the long diameter of the centrosome is parallel to the direction of its movement. This is shown in Figures 33, 34, 35. It is also noteworthy that, in the revolution of the spindle, its poles (centrosomes and asters) always seem to precede, while the other parts — mantle fibres, spindle fibres, and chromosomes — lag behind. This suggests very strongly that it is the centrosome which is the originator and director of this interesting change in the relative positions of the various parts of the cell. In this, as in numerous other instances of cell activity, this small body appears indubitably to act as the directive or dynamic centre of the cell.

By the time the rotation of the spindle is completed the centrosome and other structures at the centre of the aster have changed considerably in appearance. The centrosome is always at this time of a distinctly dumbbell shape. It consists of two aggregations of centroplasm connected by a bar of the same material, as shown in Figure 50 *b*. Very often this connecting bar or thread is relatively of considerable length,

and is also frequently more or less curved, forming structures which resemble quite closely the centrosomes figured by Boveri (:01). At about this stage (late metaphase) the centrosphere reaches the height of its development. It is usually at this time larger than at any other period of cell division and is quite definitely marked off from the other elements of the astral system. In many of the cells of this stage, as in those preceding, some at least of the astral rays can be seen to pass through this area and come into contact with the centrosome. This can be seen only in those cells which have been decolorized more than usual, but it probably exists in all well fixed material of this species. In the late metaphase the centrosphere, which up to this time has been of an oval shape (Fig. 50 *a*), also shows evidences of the approaching division. There appears in its middle region a constriction which proceeds until the structure in question assumes a distinctly bilobed form (Plate **4**, Fig. 51; Plate **8**, Fig. 146; Plate **9**, Fig. 148). This change is followed by the division of the centrosome and the further constriction of the centrosphere. At this period we have at the centre of the aster two masses of archoplasm (centrospheres) which are nearly perfectly spherical in shape, but still adhere to each other upon one side. In the centre of each of these spheres is a small darkly stained granule of irregular shape, the centrosome.

Thus in the anaphase is completed the division of the centrosome, the first indication of which was made apparent in the mid prophase. From the length of time required to accomplish this division, as well as from the great variety of stages exhibited, we should seem to be justified in concluding that it is of importance, and that the division is an accurate one as regards bulk at least. Such, however, does not always appear to be the case. In Figure 50 *b* is shown a stage of centrosome division in which the two lobes are apparently very unequal in size. Whether such a figure would eventually result in an unequal distribution of the centroplasm or not, I cannot say, but I have seen no completely divided centrosome in which the two parts are markedly unequal. It is possible that the substance is later rearranged so as to result in an equal division.

Another point to be noted in considering the centrosome is the irregular form which it exhibits at various stages. This would seem to indicate that this body is not a simple granule, as it is often considered, but that it is composed of an aggregation of granules. This would, I believe, serve to distinguish it further from the centriole as reported in many other kinds of material, as this structure is, I believe, almost universally said to consist of either one or two simple granules.

### B. *Division of the Second Spermatocytes.*

At the completion of the first spermatocyte division the two resulting cells have the appearance represented in Figure 39 (Plate 3). The divided centrosomes lie close together and are in close contact with the cell membrane at the pole of the cell opposite the remnants of the spindle. The centrosome is surrounded by no distinct centrosphere, although there is usually a small area of unstained hyaloplasm about it, similar to that seen in the preceding prophase. The astral rays have nearly all disintegrated, and only a few short indistinct ones still radiate from the centrosomes.

The nucleus, which is much smaller than in the first spermatocytes, is situated at about one fourth the distance from centrosome to Zwischenkorper. During the second spermatocyte prophase the chromosomes undergo but little change. The most striking thing about them is the fact that the number of chromosomes in the two cells resulting from the same primary spermatocyte is unequal. This inequality of division is due to the peculiar character of the accessory chromosome. As we have seen, this element is derived directly from one of the spermatogonial chromosomes, and during the first spermatocyte prophase possesses a dyad structure, while all of the rest of the chromosomes are tetrads. During the first spermatocyte metaphase this peculiar element may be recognized in most cases, though not in all.

It is always of the shape represented in Figure 40, *chr'so. acc.*; but this characteristic alone is not sufficient for its recognition, as the ordinary tetrads may also be of this form. However, there is one characteristic which, although rather illusive and often difficult of recognition, nevertheless almost invariably serves to differentiate this element from the others. This characteristic consists in the method of its attachment to mantle fibres. The ordinary chromosomes are connected with both poles of the spindle, but the accessory chromosome is connected with only one astral system in this manner. In Figure 40 I have shown this condition as it exists in four cells which are the products of two spermatogonia. These drawings were made with the greatest care by the aid of a camera-lucida, and only after very careful study of the chromosomes and their relation to the fibres of the spindle. Often the accessory chromosome is seen to be attached to the one pole by two mantle fibres, and these are inserted into opposite ends of this element. When the rest of the chromosomes divide and separate, during the anaphase, the specialized chromosome passes to one pole undivided, and thus is it brought about

that one daughter cell contains seventeen chromosomes, while the other possesses but sixteen (Figs. 39, 41).

At the opening of the prophase of the second spermatocyte the sixteen ordinary chromosomes are of a granular consistency, although their form is so definite that there can be no question of their individuality. They have the appearance of short rods of diffuse chromatin the centre of each of which is slightly constricted, thus producing a dumbbell-shaped body (Figs. 41, 42). In the succeeding stages these become more dense, and finally go to the equatorial plate as homogeneous bodies of a distinctly bilobed form. The chromosomes are rather closely crowded within the small nucleus, and there does not seem to be any large amount of linin. When arranged in the equatorial region (there is no true equatorial plate any more than in the first division), the lobes of these bodies are directed toward the poles of the spindle, thus giving basis for the conclusion that we have here a cross division of the chromosome.

Meantime the changes occurring in the archoplasmic structures incident to the formation of the spindle are rather unusual. The centrosome with its surrounding asters does not leave its position upon the cell membrane, but separates and moves apart along this structure. As the migration continues (Fig. 41), the surrounding astral rays become more numerous and at the same time more definite, and they extend farther into the cytoplasm. During the progress of this migration the cell wall in the region of the centrosome is pushed outward, possibly by the growth of the astral fibres (Fig. 41). This process continues until the astral systems have reached points upon the cell membrane at opposite poles of the nucleus, so that a plane drawn through the two centrosomes would bisect this vesicle (Fig. 42). At this time the astral rays upon the side of the centrosome nearest the nucleus are continuous with the similar rays from the other centrosome, thus forming a spindle the rays of which pass over the still persisting nuclear membrane. When the nuclear membrane finally disintegrates, the centrosomes, still close to the cell membrane, have arrived at opposite poles of the cell, and the rays extending from them penetrate all portions of the cytoplasm. Those which were seen to be continuous and to pass over the nuclear membrane become, upon the disintegration of this structure, attached to the chromosomes and serve as mantle fibres. Those of the two astral systems which proceed from the centrosomes in other directions remain distinct. This is very well shown at the stage represented in Figure 43, where those from the two poles, proceeding in a line diagonal to the long axis of the spindle, cross in the equatorial region and can be readily

traced to the cell membrane itself. It is surprising how clear and distinct the archoplasmic structures are at this stage, when we remember that during the preceding telophase much of the archoplasm was lost by the destruction of the spindle remnants at the time of the rotation of the cells (Fig. 41).

During the prophase the alterations in the character of the cytoplasm are very similar to those already observed in the first spermatocyte. At the beginning of this division the cytoplasm shows the same reaction to stains due to the dissolution of the astral rays and their deposition in the hyaloplasmic areas, for while the material composing the spindle remnants is lost to the cell, that derived from the astral systems is of course retained. During the course of the prophase we see the same progressive change in the staining reaction of the cytoplasm as in the first maturation division, and this progresses at the same rate as the formation of the astral rays. This is clearly seen when we compare Figures 41, 42, 43. In Figure 42 that part of the cytoplasm not yet involved in the astral systems shows the dense close-meshed structure characteristic of all the cytosome at an earlier stage (Fig. 41), while the part in which the astral rays are to be seen is much clearer. In the metaphase (Fig. 43) all of the cytoplasm is transparent and has resumed its usual reticular appearance.

During the prophase the cell is always of an oval shape (Figs. 41, 42), with the long axis parallel to that of the follicle, but at the time of the beginning of the metaphase the outline is more nearly spherical, and very often the shorter diameter of the cell is that connecting the two centrosomes. This is rather extraordinary, for, as we shall see presently, the cell later elongates in the axis of the spindle (Figs. 44, 45).

We have seen that, at the beginning of the metaphase, the dumbbell-shaped chromosomes are arranged with the two lobes directed towards opposite poles of the spindle (Fig. 43 and Figs. D, E, F). At this stage, however, one of the chromatic elements does not show the shape characteristic of the others, but is very evidently a rod split in the opposite direction, i. e. longitudinally. This peculiarity was also seen in the preceding prophase, where the accessory chromosome is of the same shape as in the first spermatocyte prophase. As seen during the early metaphase, this element is arranged with the plane of cleavage at right angles to the spindle axis (Fig. D), but upon the contraction of the mantle fibres, which are attached to opposite ends of the element, it revolves through an arc of ninety degrees (Figs. E, F.), and the component chromatids as they are pulled apart glide over each other in the manner

already noted as characteristic of the ordinary chromosomes during the first mitosis. The behavior of the other elements is quite different. These are arranged with their long axes parallel to that of the spindle, the separation of the chromatids occurring along the equatorial plane at the place of constriction. This very evidently accomplishes a cross division of the chromosome.

At the beginning of the anaphase the cell becomes lengthened along the axis of the spindle. This elongation is useful in two ways. It aids in

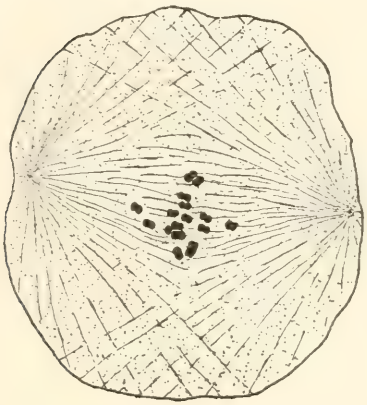

Fig. D.

Fig. F.

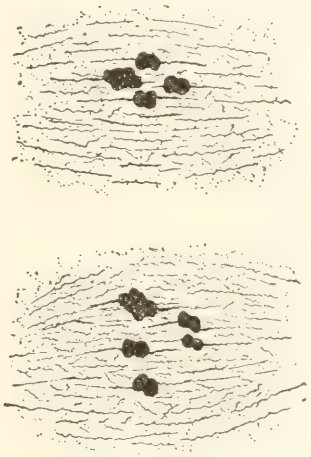

Fig. E.

Fig. D. Metaphase of second spermatocyte. The difference in shape and orientation existing between the accessory and the other chromosomes is evident. × 960.

Fig. E. High magnification of same stage, showing the differences between the accessory chromosome and the other chromosomes, in the relations of chromatids and in the attachment of mantle fibres. × 1,920.

Fig. F. Slightly later stage; showing the effect of the contraction of the mantle fibres on the orientation of the accessory chromosome. × 1,920.

the division of the chromosomes, and is also of great advantage in the later division of the cytosome. During the early stages of chromatin division the mantle fibres apparently do not shorten at all. The pulling apart of the chromosomes is accomplished by the separation of the centrosomes and the elongation of the cell, while the relative position of centrosome and chromatid remains about the same (Figs. 43, 44). Later,

however, in the anaphase, the fibres connecting the chromosomes and centrosomes do shorten, and the result is that the chromosomes are drawn toward the poles, and at the same time become apposed to each other and form a compact mass. The centrosomes during all the changes after their division in the anaphase of the first spermatocyte remain distinct and at no time show any signs of another division. They pass over into the spermatid in this simple condition as small deeply stainable spherical granules.

I have said that during the later stages of mitosis the cells elongate very much. This is so marked that in the telophase the length of the cell is often as much as four times the average width. As elongation continues, a constriction of the cytotheca appears in the region of the equator of the cell. This proceeds very much as in the first spermatocyte, and results in two cells, the dividing walls of which are independent. These cells are united, as in the preceding division, by a bundle of persisting spindle remnants, but here the structure is very poorly developed and persists a very short time, being often destroyed by the rotation of the daughter cells.

By this division there arises from each second spermatocyte two spermatids. These are rather large cells of an irregular oblong shape, containing a relatively small nucleus. This nucleus is at first surrounded by a very delicate membrane; later, however, as the cell proceeds in its growth, this becomes more marked. The chromatin at first is in the form of definite bodies (the chromosomes), but later these become granular without definite outline. When this is accomplished it is seen that in one half the cells one of the chromosomes does not lose its homogeneous character, but continues to stain as in the metaphase. This is the accessory chromosome, which up to the anaphase of the second spermatocyte was peculiar by reason of its dyad characteristics, while the other chromosomes were plainly tetrads. During the first maturation division this modified element has passed over undivided into one of the daughter cells, while in the second division its component chromatids have been separated. It is quite evident that by this unequal distribution of the chromatin two distinct kinds of spermatozoa necessarily arise. This phenomenon has recently been reported by several investigators of arthropod spermatogenesis, and several theories have been founded upon this fact. I will not enter upon these here, but later, in the discussion of the literature, will give some account of them.

### 5. Maturation Divisions in the Small Spermatocytes.

I have shown that the cells of the type of small spermatocytes are distinguished in the vesicle stage from those of the large type by the following characteristics: they are much smaller, the diameter being but about one half that of the larger type; they are not connected in pairs by a persisting spindle, each cell being entirely distinct; they are not arranged in any regular manner in the follicle, since they are so crowded that any regular arrangement is impossible; they are so forced together (Plate 5, Fig. 56) that their outlines become polygonal (i. e. they are more like ordinary testicular cells). They also differ from the large type in the relative sizes of the different parts, being characterized by a smaller amount of cytoplasm and archoplasm and a relatively greater amount of nuclear material. These discrepancies, if unaccompanied by differences in the later behavior of the cells, would not be sufficient to warrant their treatment under a separate heading. However, as the subsequent differences are even greater than those in the vesicle stage, I believe such a division is necessary for the more perfect understanding of the spermatocyte changes.

These cells offer an excellent example of the effect which an apparently small difference in the surrounding conditions may exert upon slightly differentiated cells identical in origin and early behavior. The conditions up to the time of the formation of the karyosphere are apparently identical (Fig. 1). After this, however, the conditions around part of the cells show a considerable change, which seems to exert an immediate and powerful influence upon the further development of these elements. Later (viz. in the second spermatocyte) the conditions around the cells of the two types again become similar, and their behavior is then practically the same, notwithstanding the difference in size of the cells and the small difference in structure.

The germ cells of this animal are very slightly differentiated from the typical cell, and seem to be in a very plastic condition, since they are apparently easily acted upon by external conditions. This is shown not only by the separation of the spermatocytes into two types, but also by the behavior of the abnormal cells described on a preceding page. This plasticity of the cells is at all times very striking; indeed the extent to which the behavior of the cell is modified by seemingly slight influences is often nearly incredible.

We have seen that in the spermatocyte of the first type the first change noticeable in the prophase concerns the centrosomes and archo-

plasm. Such is also the case in the smaller spermatocytes, but the change which occurs is entirely different. In these, as the centrosome moves toward the nuclear membrane, the archoplasm, which has heretofore been arranged in a zone surrounding the nucleus, now becomes entirely collected upon one side of this vesicle (the side occupied by the centrosome, Fig. 57). This mass of reticular archoplasm does not become dissolved at once in the hyaloplasm (Fig. 58), as in the large spermatocytes, but, at the time of the migration of the centrosomes, gradually disintegrates (Fig. 59) and becomes converted into the astral systems surrounding these bodies. Fragments of the archoplasm are often present in the cell up to the time of the dissolution of the nuclear membrane.

In the very early prophase, before the centrosome has left the mass of archoplasm and moved to the nuclear membrane, the nucleus already shows signs of activity (Figs. 57, 58). The chromatin segments arise from the karyosphere and go through stages similar in a general way to those in the larger type. However, there are some slight differences. The process of tetrad formation requires much less time than in the large type, and by the time the centrosomes have begun their separation along the nuclear membrane, this process is nearly completed (Figs. 59, 61). The segments as they arise from the karyosphere are much shorter and thicker than in the larger spermatocytes (Fig. 58), and this fact causes the resulting tetrads to resemble much more the typical structures seen in the spermatocytes of insects. As in the large cells, the tetrads are of diverse form, but are all referable to one class, of which the cross-shaped figure is the type (Fig. 60, *a*). The more common variations of this type are the double-V figure as shown in Figure 60, *a*. It can be readily seen how these forms are derived from crosses by a comparatively slight alteration. In the ring figure the long arms of the cross are bent around and come into contact, thus forming an apparently closed circle, while at the point of double fission the ends of the four chromatids are easily distinguishable (Figs. 58, 60, *b*). During the later stages of chromatin transformation the tetrads shorten and become condensed into homogeneous bodies which are normally of a four-lobed form (Figs. 60, *c*, 62–64). However, as in the other type of spermatocyte, there are variations from this typical form, due to slight natural modifications and distortions (Fig. 60, *c*). When fully formed it is seen that the chromosomes of the small spermatocytes are slightly smaller than those of the large type, but when the size of the cells is considered they are relatively larger.

At the time when the quadripartite chromosomes are completely formed, but are yet of a granular consistency, the archoplasm exhibits its first marked activity (Fig. 59). Previous to this, it had become rearranged in a denser mass upon one side of the nucleus, and the centrosomes had moved through this mass toward the nucleus. Now, however, these structures undergo more striking changes. The mass of faintly staining archoplasm which has surrounded the centrosomes since the telophase of the spermatogonium loses its staining capacity, and the centrosomes now seem to be enclosed in a vacuole of hyaloplasm (Figs. 57, 58). The sphere containing the centrosomes moves toward the nucleus, and as it passes through the archoplasm, this substance is reorganized and converted into astral rays. When the centrosomes reach the nucleus, they immediately begin to move apart along its membrane (Fig. 59), and as this separation takes place the astral rays continue to become more marked and the reticular archoplasm gradually disappears. The centrosomes continue to diverge until they have reached points upon the nuclear membrane about 100° to 120° apart, at which time the membrane begins to disintegrate and the spindle begins to be formed. In the type of small spermatocytes I have noticed no case in which the centrosomes have reached the full distance of 180° apart, as is usually true in the majority of germ cells. The reason for this phenomenon I cannot state, but it may have to do with the greater relative volume of the chromatic structures in this type of spermatocyte. I have never observed it in the larger cells.

Another peculiarity of the behavior of the cell at this time concerns the dissolution of the nuclear membrane. As I announced in my first paper on Scolopendra (Blackman, :**01**), that part of the membrane over which the centrosomes have not passed in their migration is the first to be dissolved, while the remaining portion persists for some time, as shown by the numerous cells in which it is still to be found (Fig. 62). To me this seems inexplicable, and is exactly the reverse of what I should expect. The centrosomes seem to exert a powerful influence upon the archoplasm with which they are in contact; this seems to cause the archoplasm to dissolve, and later to reappear in fibrillar form. They appear, however, to have an exactly opposite effect upon the nuclear membrane, as is shown by the fact that the part of the membrane with which they have come into contact persists for a considerable time after the rest of the membrane has disintegrated. This would seem to indicate that the two structures, nuclear membrane and archoplasm, are different in their chemical properties.

During the migration of the centrosomes the centrosphere has again changed its staining reaction and now shows a stronger affinity for iron-haematoxylin. As in the larger type, however, the stain is removed when the decolorizing is continued for some time, and thus the centrosome itself is made visible. During the late prophase each centrosome is elongated and constricted at the centre (Fig. 61). It is evidently preparing to divide for the second spermatocyte mitosis.

As the nuclear membrane on the side opposite the centrosomes disappears, the mantle fibres quickly form and are seen connecting the centrosomes to the chromosomes. These mantle fibres seem quite plainly to be derived from the linin of the nucleus, although the nuclear membrane itself may also take part in their formation. As the membrane disintegrates, the linin, formerly arranged in granular rows, becomes fibrillar, and may be seen connecting the different chromosomes and uniting with the fibres centring in the centrosome (Fig. 63). Whether or not they form the entire set of mantle fibres, I cannot say, but I believe there can be no doubt whatever that they contribute to it. These conditions are plainly shown in Figures 62, 63. On account of the incomplete migration of the centrosomes, the spindle as first constructed is asymmetrical (Figs. 62, 63). The chromosomes do not at first lie directly between the centrosomes, but are off at one side. Indeed, immediately before the formation of the spindle, these elements seem to be forced toward the side of the vesicle farthest from the centrosomes. However, the mantle fibres soon contract and draw the chromosomes into the space between the centrosomes, and thus a symmetrical, though very short, spindle is produced (Fig. 64).

The central spindle is very well developed. The astral rays at the sides of the spindle are numerous, stain very distinctly, and can with ease be traced without break through the cytoplasm nearly to the cell membrane. Here they divide into several divergent branches, and some of the branches, at least, come in contact with the cell membrane. That they are attached to this structure, there can be no doubt whatever. Such certainly is the case during the metaphase in a large number of cells examined, and such is certainly true of many of these fibres in the anaphase and telophase. Beyond each pole of the spindle, between the centrosome and the cell wall, there is a cone-shaped area through which very few astral rays extend. This region is occupied by a coarse-meshed network of cytoplasm, in the meshes of which there are no stainable deposits. The astral rays in this region are not as numerous as elsewhere in the astral system and furthermore are very short.

The chromosomes of this small spindle are grouped together very closely, but their outlines remain distinct. In comparison with the size of the cell, they are considerably larger than in the spermatocytes of the large type, although in actual size they are smaller. They are ovoid or bean-shaped and very seldom show the four-lobed form characteristic of the earlier stages and of the tetrads of the large spermatocytes.

Very soon after the chromosomes are drawn into position the centrosomes move apart and the spindle lengthens until the cell presents the appearance shown in Figures 65 and 158 (Plate 9). At this time the cell resembles the ordinary male cell more than at any previous time, although even now it presents several unusual features. The chromosomes remain as in the stage last described except that they are not so crowded. The cone-shaped areas of cytoplasm at the poles of the spindle are still visible, but are less marked than formerly, owing to the fact that the astral rays are better developed in these regions. However, they are still very apparent. At this stage the fibrillar structures of the cell are more conspicuous than at any time previously. It can be seen with the greatest distinctness that the astral radiations near the equator cross each other and proceeding on through the cytoplasm, come in contact with the cell membrane, to which they probably attach themselves. These astral rays, which stand forth with clear-cut outlines, are distinctly seen to be branched near their distal ends.

The centrosphere is a rather large, finely granular body which has the form of a wedge or cone with the apex directed toward the equator of the spindle. The centrosome within it is elongated in the same direction and is also conical, although much more acute than the centrosphere.

The next change which the cell undergoes is very unusual and I believe has been reported only in Scolopendra heros (Blackman, :01). The centrosomes continue to move in opposite directions until they reach points only a short distance from the cell membrane. Here they come to rest and retain their position until the separation of the chromosomes during the anaphase. But the peculiarity about this movement is that the mantle fibres still converge to the points occupied by the centrosomes before the last part of their migration. From these points, which in my preliminary paper I called the *apical points*, small bundles of fibres extend to the centrosomes, thus preserving the connection between the spindle and the dynamic centres of the cell. That the centrosomes have not lost their function, is shown at this stage by the fact that the astral rays still radiate from these bodies. As to what is the significance of this marked modification of the mitotic figure, I will not venture a

prediction, but there can be no doubt whatever that such a phenomenon occurs (Figs. 159, 160). By these changes the cones of cytoplasm at the poles of the division figure are very much decreased in size, but are still apparent (Figs. 66, 67); indeed, they still continue to be visible up to the time of the early telophase.

The next changes which take place in the cell concern the division of the chromosomes. This is quickly accomplished, the groups of daughter elements beginning at once to move toward the opposite poles of the cell. This movement is apparently brought about, not entirely by the contraction of the fibres connecting the poles of the spindle with the chromosomes, but likewise by the contraction of those uniting the centrosome and the apical point, for in all stages succeeding the anaphase (Fig. 67), the mantle fibres, as long as they persist, centre in the usual structure (Figs. 68, 69). Each group of chromosomes, as it approaches its destination, collects into a dense mass in the usual manner, as shown in Figure 68. These masses of chromatin do not reach the centrosomes, but remain at the region which the apical point formerly occupied.

When the chromosomes have reached this point, the centrosomes again move apart and take up their final position directly in contact with the inner face of the cell membrane. By this last movement the cytoplasmic cones, visible up to this time, are of course entirely obliterated (Fig. 69). We have seen that during the metaphase and early anaphase the centrosome and centrosphere are elongated in the direction of the axis of the spindle (Figs. 54, 65–67). At the completion of the anaphase this is no longer true. When the migration of the daughter masses of chromatin is completed, the centrosphere has lost its conical shape and is spherical or oval. It is worthy of note that after the retraction of the fibres connecting centrosome and apical point the centrosphere increases very appreciably in size. This affords still further evidence of the formation of the centrosphere from archoplasmic material. When the anaphase is entirely completed, and the centrosome has assumed its final position in contact with the cell membrane, it is seen that the centrosome also has changed form (Figs. 55, 69). It is now a dumbbell-shaped body with its long axis at right angles to its former direction, i. e. it is now parallel to the cell membrane.

The phenomena connected with the division of the cell body of the small spermatocyte are similar to those already noted for the large type. However, the behavior of the centrosomes and archoplasmic rays indicate even more strongly than in the other type the fundamental influence of these structures upon cytoplasmic cleavage. I have noted above that

during the metaphase the astral rays which extend through the cytoplasm in the neighborhood of the spindle are very numerous and exceedingly well developed, much more so than those which are directed toward points nearer the poles of the cell. The latter are few and comparatively inconspicuous. Each of the stronger fibres as it approaches the equatorial region divides into several branches, slightly finer than the main fibre, but still very definite and easily differentiated from the cytoplasmic reticulum. These branches proceed through the dense outer layer of cytoplasm and may be distinctly seen to come into contact with the cell membrane. I believe there can be no reasonable doubt that they attach

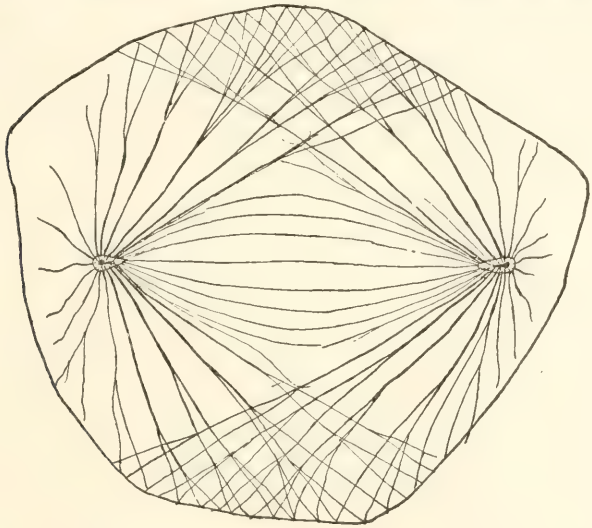

FIG. G.

**FIG. G. Diagramatic representation of a few of the astral rays in the metaphase. The fibres are much branched and cross in the equatorial region. The branches come in contact with the cell membrane and probably are attached to it.**

themselves to this structure. I have never seen instances where the peripheral astral rays from one pole unite and become continuous with those of the other as is apparently the case with the spindle fibres. On the contrary, it can be definitely seen that they behave in quite a different manner. Each ray in the peripheral equatorial region is sharp and distinct, and in sections parallel to their course can often be traced from their origin in the centrosome to their points of insertion in the cell membrane. It can be very plainly demonstrated that during the meta-

phase, anaphase, and early telophase the rays cross each other in the equatorial region and proceeding onward in a straight line attach themselves to the cell wall beyond, as shown in the accompanying text figures, which are diagrammatic drawings of actual cells.

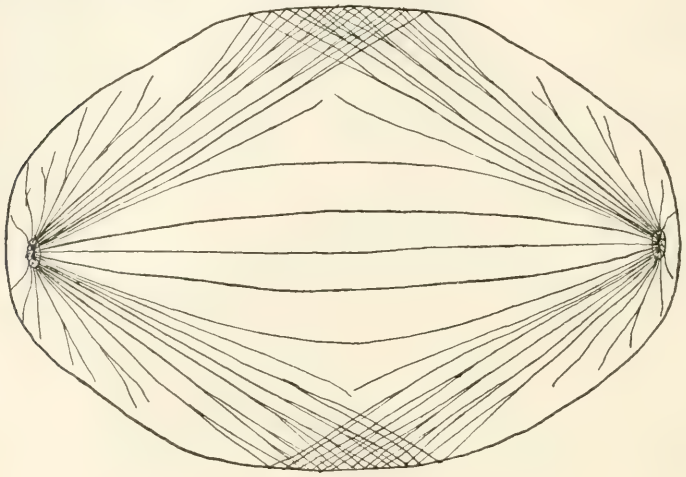

FIG. H.

FIG. H. Diagram of the astral rays in the anaphase. The branching of the fibres has partially disappeared owing to their coalescence to form thicker fibres. The region of attachment upon the membrane is more limited.

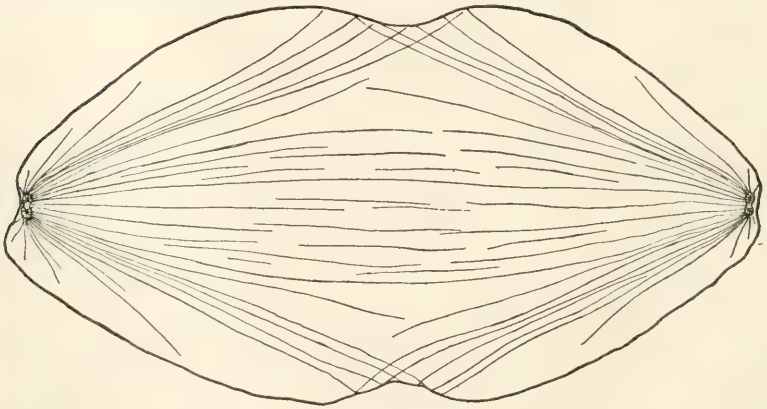

FIG. I.

FIG. I. Diagram of the astral rays in the early telophase. The branching has entirely disappeared and the constriction of the cell wall has begun. The area of attachment of the fibres is still smaller and is limited to the region actually undergoing constriction.

In the metaphase the astral fibres found in the equatorial region are very much more numerous than elsewhere and are much better developed. Their place of attachment upon the cell membrane is confined to a rather limited zone about one third the cell diameter in width. During the subsequent lengthening of the cell in the anaphase this area at first remains practically the same, but later as the elongation proceeds becomes still more restricted to probably one half of its former width. Thus, at the time of the constriction of the cell membrane, the attachment of the greater number of the astral rays is confined to the small area of the cell wall with which this process is chiefly concerned, i. e. the equatorial portion only. Those which are attached to the cell wall at the points between this area and the poles of the cell, are very few and very faintly developed; furthermore they are so arranged that, practically, they can exert by their contraction no considerable force upon the cell membrane. This is owing to the fact that as the cell lengthens the polar regions become cone-shaped, and the centrosome, being at the apex of this cone, the force exerted by the contraction of these rays acts approximately parallel to the cell membrane, so that it can have no appreciable constricting effect upon this structure. With the aid of the polar astral rays and the central spindle, however, these fibres serve more firmly to anchor the centrosomes. Soon these fibres, being no longer of any use, disappear and are seen no more.

In a slightly later stage (Fig. 68), when the cell wall has begun to show signs of constriction in the equatorial region, the centrosome again changes its position with regard to the cell membrane, as already described. The cell wall at the point of contact with the centrosome is drawn inward, thus causing a considerable depression in this region (Fig. 69).[1] This depression shows that the centrosome is attached to the wall at the poles, and that the force necessary to complete cell division is obtained, not by the divergence of the centrosomes, as was apparently the case in the separation of the chromosomes, but by a force exerted through the astral fibres.

Now a few words in regard to the significance of these phenomena. I have noted above that during the metaphase the astral rays which, extending through the cytoplasm, envelop the spindle on all sides, plainly come in contact with the cell wall and very probably are attached to it. That these rays, which are by far the most numerous and best developed of those constituting the asters, should do this to no purpose, is

[1] The same appearance is also noticed, though to a less extent, in the first spermatocyte of the larger cells (Plate 3, Figs. 37, 38).

incredible. From their situation they can in no way aid in the separation of the chromosomes. It is, however, very evident that they may be of material use in the later phenomena of cell division. Indeed, it can be plainly seen that they may be of use in the elongation of the cell during the anaphase. As the chromosomes move apart the centrosomes also diverge, but still maintain a fixed distance from the cell walls at the poles of the cell. This is accompanied by an elongation of the cell. I believe that this lengthening of the cell is accomplished in the following manner. The centrosomes are united with the cell membrane in the equatorial region by means of astral rays. As they move apart (influenced probably by some repellent force which they exert upon each other, or possibly by the action of the central spindle), the rays which are inserted in the cell membrane at the equator are put under greater tension, and therefore pull with greater force, than rays farther away from the axis of the spindle. This naturally lessens the diameter of the cell in the equatorial region and also necessarily causes the diameter corresponding with the axis of the spindle to increase proportionately. With the elongation of the cell the centrosomes come to lie upon the cell membrane, and the force exerted then by the astral rays which are attached farthest from the equator comes to act approximately parallel to the cell membrane, and thus the power which they exert upon this structure is minimized. This condition is most obvious in those cases where both ends of the cell have become conical.

During the metaphase the fibres attached at the region of the poles are finer and less well developed than those nearer the equator. As the equatorial diameter diminishes, the cytoplasm formerly in this region is forced away, and moves to the ends of the cells, thus causing the elongation of the cell. Of course, this also has the effect of further lessening the force applied at the pole, because the number of rays is relatively less for the amount of cytoplasm. However, the force exerted by the fibres which are attached in the equator is still applied at a sufficient angle to cause a further lessening of the cell diameter at the point of application.

It is, I believe, the universal conception that the cell membrane is a flexible, elastic structure, which is capable of being much altered by very slight irregularities in the forces acting upon it. When a force applied at several points of such a structure acts in the direction of any fixed point outside the membrane, the effect is to cause the points of application to approach one another. Thus, when the astral rays attached in the equatorial region become active, the effect is for the

points upon the membrane at which these rays are inserted to come together. As the traction is exerted from opposite poles of the cell, the forces are so equalized that the place of this concentration of the cell membrane naturally falls in the equatorial region. Thus it comes about that the stress upon the cell membrane at this place is much greater than at any other. The attachment of the fibres being thus limited to a very small area, it is evident that the traction thus exerted would cause an annular constriction of the cell membrane at this plane. Were the force exerted from only one point, the contraction of the connecting rays would cause the membrane to be drawn toward that point. However, this is not the case. The power proceeds from two sources, which are at equal distances from the point of application and are attached at equal angles to the membrane. As the fibres proceeding from one of these sources form an obtuse angle with those proceeding from the other, the effect of contraction is to cause the two fibres to extend in a straight line between the two points. As this proceeds, the cell membrane, attached firmly to these rays, is drawn inward, so that, at the time when the two groups of fibres become parallel to each other, the cell membrane has constricted until there is but a very small opening connecting the two lobes.

We have seen that during the constriction of the cell there has appeared a depression in the membrane at the point where the centrosome rests upon its surface. This phenomenon I believe can be explained as follows: During the lengthening of the cell it appears that the required force probably is produced by a repulsion exerted by the centrosomes upon each other, causing them to separate. This explanation is no longer sufficient for the subsequent changes, as the centrosomes no longer continue to move apart. Therefore the later changes must be accomplished by the contraction of the archoplasm fibres. That this is true is shown by two facts: the observed thickening of the fibres, and the depression in the cell membrane at the place of attachment (polar region).

While the constriction of the cell wall is taking place, the interzonal filaments extending between the two daughter masses of chromatin apparently take no active part, although they may well play a passive one. During the later stages the ends nearest the poles seem to lie free in the cytoplasm and to have no connection whatever with the centrosomes. It is evident that even at this early stage of the telophase, shortly after constriction has begun, these structures are beginning to disintegrate, for the distal ends — i. e. those nearest the poles — have become granular

or have even disappeared. As the constriction proceeds, the filaments are forced inward in the form of a bundle, the density of which continually increases as the diameter becomes less and less. The free end of this bundle has a tendency to retain its old position in the cytoplasm, so that in the later stages some of the fibres are curved outward toward the cell membrane, the ends being much farther apart than the central portion (Plate **5**, Fig. 70). It can readily be understood that this arrangement of the interzonal filaments obscures very much the astral fibres, which are still active. The majority of these, at the time the cleavage furrow has nearly reached the centre of the cell, extend in a straight line from the bottom of this furrow to the centrosomes, but the number of filaments is so great that this arrangement is considerably obscured. The "Zwischenkörper" thus formed does not persist very long, but soon disappears, probably as a result of the rotation of the cells upon each other, which seems always to occur during the telophase. In Figure 70, where the constriction is as yet hardly complete, this rotation has apparently already begun.

These conclusions concerning cell division, while by no means new, contain several new features. Every point is based upon actual observations made upon different stages in the process of mitosis, and I believe the conclusions drawn from these observed facts are correct. As regards the source from which the power requisite for cell division proceeds, it is necessary to conclude that it proceeds either from the centrosome, from the archoplasm, or from the two working together and in harmony. It is necessary to say that during these phenomena the centrosome acts at least as the directive centre. During the earlier stages of actual division (metaphase and anaphase) the separation of the chromatin masses and elongation of the cell are accompanied by the moving apart of the centrosomes. This divergence must be accomplished, I believe, either by the activity of the centrosomes themselves, or through some force which is exerted upon these organs by the archoplasmic fibres. The only part of the archoplasm which could accomplish this is the central spindle. To be sure, the cell membrane and centrosome could be brought together by the contraction of the astral rays in the polar region. But it is readily seen that this would not have the observed effect. The contraction of these structures would cause the membrane to approach the centrosome and the cell would be elongated in the plane of the equatorial plate. Then, in addition, these polar rays are less developed than any others of the cell The lengthening of the cell occurs at right angles to the equatorial plane,

i. e. parallel to the axis of the spindle. Thus it is evident that the phenomena must be explained in another manner.

During the separation of the centrosomes the chromosomes are also drawn apart into two groups, and there is seen between these two groups of elements a spindle-shaped structure composed of well-developed archoplasmic fibres. During the elongation of the cell this structure also elongates and at the same time widens considerably (Figs. 68–69). The spindle being placed between the centrosomes, if it exert any force upon these elements resulting in their further separation, must do so by a process of elongation or growth. It is very improbable, I think, that sufficient force could be exerted in this manner to accomplish the elongation of the cell which invariably ensues. I know no single instance in animal tissue in which any large amount of work is accomplished by the lengthening of a fibrillar structure. The reverse is invariably the case when any force is required, for example, the contraction of the fibrillae in muscle cells.

Then, it is very probable that the divergence of the centrosomes is accomplished by some force centring in them, i. e. that, while they attract other parts of the cell, they exert a mutually repellent influence upon each other. That organs so very small should possess such great power will not be admitted by many, yet I believe no better explanation is now at hand. The potentiality of these minute structures is shown at various times in the cell's development: in the early prophase, when the nuclear membrane is drawn out into a conical protuberance (Plate **2**, Figs. 20, 22); later, when it is forced inward (Figs. 21, 29, 30); at the time of the disintegration of the nuclear membrane, when the linin fibres are drawn toward, and centre in, this body (Figs. 30, 31; Plate 4, Figs. 48, 49); in the metaphase of the large type, when the spindle revolves through an angle of 90° (Plate **3**, Figs. 33–35); in the metaphase of the small type, when the chromosomes are drawn to a position between the centrosomes (Plate **5**, Figs. 62–64). At all these stages, as well as in the separation of the chromosomes and the cleavage of the cytosome, the centrosomes show their great influence over the other cell structures. The importance of this cell organ is also shown by its presence at all phases of mitosis and at all stages of cell development. That these structures should behave as they do and still be of no fundamental use in the cell, is incredible. At all periods of development the centrosome shows by its behavior that it is of great importance in the cell's economy and that it is indeed one of the most important organs of the cell. That it is probably an organ of equal rank with the

nucleus is shown by the recent experiments of Wilson (:01[b]) upon etherized sea-urchin eggs.

The later changes of the telophase — the disintegration of the chromosomes and the formation of the nuclear membrane — are apparently identical with those of the large type of spermatocyte. Indeed, most of the changes in the succeeding division are quite strikingly similar to those already described in the latter cells. This fact would at first seem remarkable, when the dissimilarity of the earlier stages is considered, but, as I have said, these cells at all stages seem to be in a plastic condition and very much under the control of surrounding influences. The environmental conditions of the two types of spermatocytes during the second spermatocyte stage are similar, the only discrepancies apparently being a difference in the size of the cells themselves.

The earlier changes of the prophase are so similar to those occurring in the large type, that I think it hardly worth while to describe them again in detail. The dyads arise in the same manner from the slightly diffused chromosomes characteristic of the late telophase; and the centrosomes, after migrating apart along the cell membrane, take up positions upon the cell membrane opposite to each other (Fig. 71). The nuclear membrane is usually not re-formed, and the chromosomes are drawn into the spindle where they are irregularly placed (Fig. 72). They are often apposed to each other in such a manner that they seem to be in the form of one or several convoluted bands. However, there is certainly no real union of the chromatin during the second spermatocyte stages, for by careful focussing the outlines of the individual elements can always be clearly seen.

In shape and general characteristics the spindle resembles very closely that of the large cells. The astral systems are well developed, but while the rays are numerous, they are not as markedly so as in the former type. It is noticeable, however, that the rays are arranged differently from those in the first type, and this may indeed account for the slight difference in the subsequent cleavage. As in the first spermatocyte, they cross in the equatorial region, but are attached to the cell membrane in a very limited area. This brings it about that cleavage takes place without that extraordinary elongation of the cell characteristic of the large type of the second spermatocyte. For, whereas in the small spermatocyte the rays in the early metaphase are already grouped in the plane of constriction, in the large type this grouping must be accomplished by a further elongation of the cell in the manner already described.

The cytoplasmic cleavage of the cell is accompanied by the same phenomena as in the first spermatocyte. The midbody is poorly developed and persists but a short time (Figs. 73, 74). Upon its disappearance the cytoplasm takes on the dense stain which has been noted as characteristic of the prophase of the first spermatocyte during the formation of the astral systems. The archoplasm is again dissolved, but is later converted into irregular reticulated masses, the presence of which characterizes certain stages of the spermatid (Figs. 75, 76). Meanwhile the centrosome, which has remained upon the cell membrane since the completion of division, leaves this peripheral position and comes to lie in one of these masses of archoplasm (Fig. 76).

In the telophase the chromosomes go through the usual changes. They have become granular and are now in the form of a number of flaky masses of chromatin, arranged irregularly throughout the nuclear space, which at this stage is surrounded by a delicate membrane. As in the large type of cell, some of these spermatids possess the accessory chromosome while in others it is lacking. After careful count it is found that the cells possessing this element are about equal in number to those in which it is lacking. This element has been divided in but one of the preceding divisions, and during the other division has passed over bodily into one of the resulting cells.

### 6. The Metamorphosis of the Spermatids.

In studying the metamorphosis of the spermatids of Scolopendra heros most of the observations here recorded were made upon spermatids derived from the larger spermatocytes. However, sufficient study of the smaller cells was made to warrant the statement that the process is nearly the same in both, and that the results of the transformation of the smaller spermatids are spermatozoa identical with the larger ones in all particulars except size. The behavior of the cells of the two sizes is so similar, and the results are so nearly identical, that one is forced to the conclusion that the spermatozoa of the two classes are equally functional in the fertilization of eggs. This conclusion is also strengthened by the similarity of behavior in all fundamental particulars of the spermatocyte chromosomes of both sizes of cells.

As a result of the two spermatocyte divisions, there are derived from each primary spermatocyte four spermatids, two of which differ from the other two in the possession of an extra chromatic element — the accessory chromosome. In the telophase of the second spermatocyte this can

be demonstrated only by counting, owing to the fact that the difference in size between the specialized element and the other chromosomes is too small to serve in identification. However, the chromosomes are so distinct that accurate enumerations can be readily made (Plate **6**, Figs. 77, 78). In later stages of the early spermatid, the accessory chromosome again becomes for a time morphologically distinguishable by means of the same characteristic by which we were able to identify it during the spermatocyte changes, i. e. its retention of the homogeneous condition at the time when the other chromosomes have again resumed their granular appearance. However, this does not long serve as a means of identification, for at the beginning of the condensation of the nucleus of the spermatid into the head of the spermatozoön the accessory chromosome again becomes indistinguishable. The appearance and relations of the various parts to each other during the telophase of the last spermatocyte division are shown in Figure 77. The centrosome, one in each of the two prospective spermatids, lies at the pole of the cell opposite the remnants of the spindle. It consists of a simple spherical granule, around which there is no indication of a centrosphere; however, faint astral radiations in process of disintegration are still to be seen. It lies in close contact with the cell membrane; in fact, at this and slightly later stages it seems to cause a distinct outward bulging of the membrane at the point of contact (Figs. 77, 79).

The chromosomes, either sixteen (Fig. 77) or seventeen (Fig. 78) in number, lie in a clear area or vacuole situated at a point about four times as far from the Zwischenkörper as from the centrosome (Figs. 77, 78). They are at first small homogeneous bodies, but soon become apparently larger owing to the more diffuse condition of the chromatin (Figs. 78, 79). This change is accompanied by the formation of a nuclear membrane, which appears first at about the stage represented in Figure 78. This, while thin and delicate, is very distinct by reason of the definite black or gray stain which it takes, while the cytoplasmic reticulum assumes an orange-red color in preparations stained with Heidenhain's iron-haematoxylin and Congo red.

The cytoplasmic reticulum of the young spermatids has the same close-meshed, deeply stained condition which we have already noticed to be characteristic of the same material during the early stages of the spermatocyte mitoses. This, as in the former cases, is due to the fact that the archoplasm taking part in the preceding division has been broken down and is now distributed throughout the entire cytoplasm. As we shall soon see, aggregations of archoplasm are later to be met with

in various parts of the cell, and when this occurs the present condition of the cytoplasm no longer exists, the network becoming much coarser and more transparent (compare Figs. 78, 79 with Figs. 86–94).

In the telophase of the last spermatocyte division the two daughter cells, even when incompletely separated by the constricting membrane, seem to exhibit a mutual repulsion, as shown in Figure 77; this causes them to separate from each other as far as the persisting remnants of the spindle will allow. As in the first spermatocyte division, the dark bodies which form a band around the outside of the spindle sheaf at its middle lie entirely outside of both the resulting cells. This is quite evident in Figure 77, but is even more marked at a later stage (Fig. 78). Here a great part of the Zwischenkörper is outside the cell, and in many cases at least, it is all eventually detached in a manner similar to that which occurs in the first spermatocytes (Plate **3**, Fig. 41). As a result of this process, spermatids are produced (Figs. 79 *et seq.*) which contain no trace of a true "Nebenkern" at any stage, although a structure similar to it, which by many writers has been confounded with the Nebenkern appears at a later stage. That the archoplasmic structure seen in the spermatid at the time the axial filament is forming is not the Nebenkern, is shown by the bodily casting off of the remnants of the spindle and by the fact that for a considerable period thereafter no such structure is to be seen in the cell (Figs. 78–84).

Thus the spermatid, soon after it has become free from its mate by the destruction of the connecting bridge of interzonal filaments, presents the appearance represented in Figure 79. The cytoplasm is of the fine-meshed character noted above and is entirely free of any special aggregations of archoplasm. The centrosome still retains its peripheral position and lies in close contact with the cell membrane. There is no centrosphere or other similar aggregation of archoplasm around it, but only very faint traces of the disintegrating astral rays in the last stages of dissolution.

The nuclear vesicle is considerably enlarged, although still much smaller than it later becomes. The chromatin is much more diffuse than formerly, and the most of the chromosomes are situated at the periphery of the nucleus. They have the form of ovoid granular bodies often flattened in the plane of the nuclear membrane. From these bodies very definite deeply staining granular processes extend out and connect with similar ones from other chromosomes (Fig. 79). These processes stain with the same intensity as the chromosomes and are doubtless composed, at least in part, of chromatin.

The stage represented in Figure 79 may be taken as the starting-point in the transformation of the spermatid into the spermatozoön. The first changes which occur are unusual and rather remarkable. We have already noticed that the nucleus, since its formation in the telophase of the second spermatocyte mitosis, has increased considerably in size. The membrane in all these early stages (Figs. 78, 79) is unmistakably present and quite evident. Now, however, before the usual transformation processes begin, the volume of the nucleus increases very rapidly until it is more than twice as great as in the stage just described (compare Fig. 79 with Figs. 84, 85). This is accomplished by the imbibition of material from the cytoplasm by the nucleus. The taking up of material is probably not accomplished exclusively by an osmotic interchange through the nuclear membrane, but is so rapid that the membrane seems invariably to be ruptured. Appearances similar to Figure 80 are extremely numerous in my material at this stage, and there can be no doubt that they represent a regular process which occurs in the spermatid just before the migration of the centrosome from the cell membrane toward the nucleus.

The more usual appearance is shown in Figure 80. Here the nuclear membrane has ruptured at two places, and the cytoplasm in the vicinity of these ruptures has lost its affinity for the Congo red, the cytoplasmic stain used in this preparation, and remains unstained, which is characteristic of karyolymph. This presents the appearance of a rupture of the nucleus by pressure from within and the extrusion of a part of its liquid contents; but that such is not normally the case, is shown conclusively by a study of the stages immediately preceding and succeeding the one in question. That there must be a very considerable imbibition of cytoplasmic material is shown by the great increase in size and the rapidity with which it is accomplished, as well as by the morphological appearances described. It is also very probable, as we shall see later, that other activities of the cell are influenced by this phenomenon.

In my first paper on Scolopendra I described a process of nuclear budding by which a portion of the nucleus containing one or more bodies of chromatin was constricted off, and migrated out through the cytoplasm. At that time I considered the budding a normal process. Now, after much more extensive study, I have changed my opinion and do not believe this is a normal condition, for while such fragmentation of the nuclear material is occasionally met with, it is not common. Doubtless it is an accidental modification of the process I have already described. It is probable that, owing to the rapid taking in of fluid

from the cell substance, one or more of the chromatin bodies are forced out through one of the openings in the nucleus and there form a vesicle about themselves, just as do the chromosomes in the telophase of the mitosis in many egg cells. This gives rise to such appearances as were noted in my former article and are shown in Figures 81–83 of the present paper. In Figures 81, 82, this vesicle is shown as still connected with the nucleus, and probably the material enclosed by it is later withdrawn within the nucleus of which it continues to form a part. In Figure 83 the vesicle, though smaller, is entirely separated from the nucleus, and the nuclear membrane on the side nearest it is ruptured, showing whence the "bud" is derived. Whether in such cases there is later a fusion of the nucleus and the extruded portions, I cannot say positively. This may be the case, or the bud may be extruded from the cell entirely, as I suggested in my first paper; or, finally, the substance may be dissolved in the cytoplasm. At any rate, no such structures have been observed at later stages in the spermatid.

As a result of the rupture of the nuclear membrane and the imbibition of material from the cytoplasm, the diameter of the nucleus is increased about one half. From this stage (Fig. 84) until the time when the nucleus has begun to condense and to elongate to form the head of the spermatozoön there appears to be no definite continuous nuclear membrane. There is, to be sure, a sharp line of demarcation between nucleus and cytoplasm, due to the differences in staining reaction which they exhibit, but nothing more.

In the meantime the cytoplasmic structures have undergone some slight modifications. The reticulum is considerably coarser, and the meshes more transparent, than in preceding stages in this transparency, which had not yet reached its highest point of development. This is due to the fact that the archoplasm is being withdrawn from general distribution throughout the cytoplasm and is again becoming aggregated into specialized masses in various parts of the cell. These aggregations may at first appear in any part of the cytoplasm (Fig. 84), but in later stages they are collected principally in the region of the centrosome (Figs. 85 *et seq.*).

The centrosome at the stage when it was last described still preserved its position in contact with the cell membrane (Fig. 79). At that time it was a simple spherical granule without any enveloping layer of specialized cytoplasm. Shortly before the stage represented in Figure 84, it leaves its peripheral position and migrates through the cytoplasm toward the nucleus. The centrosome during its migration through the cytoplasm

becomes surrounded by a mass of archoplasm which increases rapidly in volume, and by the time the centrosome has reached the vicinity of the nucleus is of considerable size (Figs. 84, 86). During the earlier stages of the migration the archoplasm is often of an irregular stellate shape with long processes extending in various directions, but conforming to the course of the cytoplasmic partitions. The centrosome during the migration, which from the large number of stages found seems to occupy a considerable time, becomes elongated in the direction of movement, as shown in Figure 84.

This elongation of the centrosome is in preparation for the formation of the axial filament, the first stages in the production of which appear during this migration. In Figure 85 such a stage is represented, showing, I believe, that, while the axial filament arises in very close connection with the centrosome and possesses the same staining reaction, it is not derived from the substance of this element. At the proximal end of the entire structure, i. e. the end nearest the nucleus, lies the centrosome. It is of the same shape and size as in the preceding stage (Fig. 84). At the distal end of this elongated centrosome and fused indistinguishably with it, arises a short somewhat moniliform fibre, which stains in the same manner as the centrosome and is distinguishable from it only in being at this stage narrower, less definite in outline, and tapering toward its distal end.

This compound structure is surrounded by a considerable amount of archoplasm, which in a general way is of the same shape as the enclosed parts (Fig. 85). The archoplasm takes a darker stain than the undifferentiated cytoplasm of the cell, although of the same tint. The periphery is more granular and denser than the part immediately surrounding the forming axial filament. This latter is nearly homogeneous and much less deeply stained. In other words, the archoplasm in the central portion of the mass resembles more the condition which it assumes during the early prophases of mitosis, when the astral rays are forming.

By the time the archoplasmic mass containing the centrosome and the growing axial filament has come to rest in the vicinity of the nucleus, the axial filament is of considerable length and has the appearance of a dense black thread which stands out with remarkable distinctness against the orange-red background formed by the cytoplasm. The appearance of the axial filament and its relations to the cytoplasm, archoplasm, and centrosomes at this stage are shown in Figures 86 to 89. The axial filament, while always very distinct in doubly stained preparations, does not as a rule possess at this stage a perfectly even and continuous outline.

On the contrary, enlargements occur throughout its course, and these often alternate with constrictions which, however, never amount to complete interruptions of the thread. An extreme case of this condition is shown in Figure 89, where the fibre is typically moniliform. The other extreme exists in the cell from which Figure 86 was drawn. Here the axial filament, with the exception of its extreme proximal end, is clear cut and of uniform calibre.

The archoplasmic mass always envelops the proximal end of the axial filament, and in many cases is at first so elongated as to enclose the greater part of it. Before the formation of the filament has proceeded very far, however, its distal end extends beyond the archoplasm and stretches out through the cytoplasm toward the cell membrane (Figs. 86, 87). As a rule its course is at first tangential rather than perpendicular to the surface of the nucleus. The filament seems to have no fixed relation to the reticulations of the cytoplasm, appearing sometimes to follow the walls of the meshes, sometimes to pass through the meshes of the network (Figs. 86–89). The distal end of the fibre may be either embedded in the substance of the reticulum (Figs. 87, 88) or lie free in the hyaloplasm (Fig. 89). In Figure 87 the part of the cytoplasmic reticulum which is a direct continuation of the distal end of the filament, is somewhat more dense than neighboring parts of the reticulum, and the same is true to a certain extent in Figure 88. However, after careful study of many cells at this stage I feel confident that the growing axial fibre shows no constant relation to the cytoplasmic reticulum.

Now, as regards the relation of the centrosomes and axial filament. When these structures have reached the vicinity of the nucleus, the centrosome has given rise by fragmentation to from three to seven deeply staining granules, which seem to bear a fairly constant relation to the proximal end of the filament. In perhaps ninety per cent of the cells examined the number of centrosome fragments found detached from the axial filament was only two. Of the remaining ten per cent only a very few cases exhibited more than three. When there are only two centrosomes they bear a constant relation to the axial fibre as shown in Figures 87, 88, 90, 93, 94, and in numerous later stages. They are almost symmetrically located on opposite sides of the fibre. Very often this end of the axial filament is considerably enlarged, and this I interpret as evidence that a portion of the centrosome still retains its direct connection with the filament (Figs. 86, 87, 88, 90 *et seq.*). As we shall see later, these conditions persist throughout nearly the whole period of transformation, being seen even in the nearly mature spermatozoön.

This arrangement is the one most commonly met with, occurring, as it does, in a large proportion of the cells at this and later stages. It is evident that sections cut in planes other than that of the three parts of the centrosome might give rise to different appearances. In many cells the plane of the section is perpendicular, or nearly so, to the plane just defined, so that in focussing one encounters, in succession, a centrosome, the proximal end of the axial filament, and the second centrosome. Figure 86 represents one of the very few cases in which the centrosomes bear a different relation to the filament. Here the joining line is nearly parallel instead of perpendicular to the filament. It is probable that in these cases the centrosomes assume the usual relation later.

In a number of cases I have observed very fine granular threads (Figs. 87, 90) that arise from the two lateral centrosomes and seem to join the axial filament at an acute angle at some distance from its proximal end. This condition is to be correlated, I think, with the disposition of the centrosomes and their relation and connection with the axial filament observed at much later periods of transformation.

In Figure 87 are shown three centrosomes distinct from the axial filament. Two of these occupy the usual position, while the third lies at an equal distance from the proximal end of the filament in the direction of its prolongation. All are connected with the filament by fine granular fibres as shown in the drawing. At the end of the filament proper there is a slight enlargement indicating the presence of a fourth part of the centrosome. In Figure 89 the centrosome fragments are still more numerous. Here there are six entirely free from the filament, three arranged as in Figure 87, two others forming a pair distal to the normal pair, and the sixth still more distal in position. These together with the knob-like end of the axial filament make the total number seven, the largest I have found.

As to the significance of this multiplication of centrosomes, I can say nothing, except that in later stages only three are present, the two lateral bodies and the central one which forms the enlargement at the end of the filament. It should be observed, however, that when a larger number of centrosomes are present they are smaller, as will be readily seen by a comparison of the figures. This is especially true of the central one, which usually is quite conspicuous, but in Figures 87 and 89 is much smaller.

The elongation of the axial filament proceeds until the distal end has reached the vicinity of the cell membrane (Fig. 91), when the lengthening of the cell itself begins. When it first appears, as we have already noted, the axial filament is usually tangential to the surface of the

nucleus. However, by the time it has grown to the length shown in Figures 91 and those following, it has become nearly perpendicular to the nuclear membrane and its proximal end is much closer to the membrane than previously. While in the process of formation the axial filament is never perfectly straight, but, on the other hand, it is never extensively curved or coiled. However, while fairly direct in its general course, it is often more or less sinuous, as shown in the figures.

Meantime the nucleus has undergone some interesting changes. At the stage last described the chromosomes had broken down into diffuse granular bodies, which were scattered irregularly over the inner face of the nuclear membrane (Fig. 79). This condition persists during the rapid growth of the nuclear vesicle. The connecting strands of linin and chromatin become even more prominent. However, at the time when the centrosome leaves the cell membrane and begins its migration toward the nucleus, the chromatin bodies become rearranged (Fig. 84). They now congregate upon the side of the nucleus which is nearest the centrosome, and retain this position until the nucleus begins to elongate to form the head of the spermatozoön (Figs. 84–93). The normal number of chromosome bodies is not always to be recognized, for in the cells containing the accessory chromosome some of the chromosomes unite with this to form a dense mass, — a karyosphere (Figs. 84, 85, 86, 93). The position of the nucleus has also changed, for it now (Fig. 91) lies upon one side of the cell a short distance only from the cell membrane.

The change which now begins is one of the most interesting in the transformation of the spermatid into the spermatozoön. The spermatid, hitherto nearly spheroidal, now begins to undergo changes of form which finally result in its extensive elongation. The first observable indication of this change of form is noticed in Figure 92, where the cell on the side farthest from the nucleus shows a wavy contour. This is true, not only of the membrane in the immediate vicinity of the distal end of the axial filament, but of the whole periphery except the third nearest the nucleus. This condition continues to become more and more marked (Fig. 93), until soon the whole cell membrane, except a small portion in the nuclear region, is involved. Projections like pseudopodia are protruded in various directions, causing the cell to assume the remarkable amoeboid shape shown in Figure 94. The "pseudopodia" are directed not only in what might be thought the most natural plane of elongation, i. e. parallel to the course of the axial filament, but in many other directions as well (Figs. 93, 94). There seems to be an attempt on the part of the cell to seek out and follow the path of least resistance (Fig. 94), the

"pseudopodia" in other regions being withdrawn, and the cell outline again assuming a more regular contour (Figs. 95, 96). This often results in peculiar shapes, the cells sometimes becoming S-shaped or C-shaped or even nearly complete rings. However, when the spermatids lie in a region of the testis where there is no considerable crowding, as, for instance, next to the lumen occupied by spermatozoa, the process of lengthening results in the formation of cells more regular in outline (Figs. 95, 96). Here the elongation has proceeded in an approximately straight line. This results in long cells containing the nucleus at one end, which by anticipation we may call anterior. From the posterior side of the nucleus, extending backward the entire length of the cell, is the conspicuous deeply stained axial filament (Fig. 95). No case has been observed in which this fibre penetrating the cell wall extended beyond the boundaries of the cell. I cannot say positively that this never occurs, but it is certain that in many cases observed both at this and later stages, the filament may be seen to be wholly included within the cell. In Figures 95 and 96 the end lies free in the cytoplasm at a short distance from the cell membrane.

The cell continues to elongate throughout all the succeeding stages of transformation (see Figs. 95–121) until, in the fully developed spermatozoön, it has reached a length of several hundred micra, and measures only 1.5 micra in diameter. In Figure 121 (Plate **7**), representing the anterior portion of one of the mature spermatozoa, the actual length of head and acrosome is 260 micra, and it is probable that the tail of such a spermatozoön measures several times as much.

As they arise from the second spermatocytes, the large spermatids always occur in groups of 8–16–24, etc. (the products of one or more pairs of first spermatocytes). This grouping is usually preserved throughout the whole period of transformation, so that at any stage a group usually consists of eight elements or a multiple of eight. The cells comprising one of these groups generally have their nuclei at about the same level (Figs. 95, 96), their elongating tails extending backward parallel to one another and in close apposition. Occasionally, when the region of the testis in which they lie is much crowded, the spermatids of a single group elongate in different, sometimes in opposite, directions, so that in later stages they no longer exhibit the characteristic grouping. This, however, is rather rare, and even the mature spermatozoa usually show the groupings into eight or multiples of eight.

When the axial filament has attained a considerable length (Fig. 91), the archoplasm is still apparently increasing in amount, and is all aggre-

gated into one mass near the nucleus at the base of the axial filament. However, at about the time when the elongation of the cell begins (Fig. 92), small portions become detached from the main mass, and some of these migrate around the nucleus and come to rest at a point directly opposite the base of the filament (Figs. 92, 93, 94 *et seq.*). This archoplasmic material, which is the first indication of the acrosome, that later becomes so prominent, has at this time the form of a number of small spherical masses. These are rather dense and granular, and are stained more strongly by the Congo red than any other structure of the cell. By succeeding changes, which are very interesting, they become altered from their granular consistency into a large vesicular structure which is characteristic of the young spermatozoa, and later into the long filament-like acrosome of the mature spermatozoön.

Apparently the first stage in this transformation is shown in Figure 98. This presents an appearance quite similar to the early stages in the formation of the acrosome in Amphiuma as figured by McGregor (**'98**); but, as we shall see, the later stages are entirely different from those shown for this amphibian. The changes in Scolopendra are as follows: Each of the archoplasmic bodies at the anterior pole of the nucleus becomes enveloped in a mantle of clear substance (Fig. 98) which has a sharply defined boundary toward the surrounding protoplasm. With the increase in thickness of this mantle, there is a gradual reduction in the size of the granular mass, until the latter has entirely disappeared and there remains in its place a clear vesicle with distinct smooth walls (Figs. 95, 96). Sooner or later these vesicles unite with one another, forming a larger and larger vesicle (Figs. 95, 97, 99). At first this is very irregular in shape (Fig. 97), and often shows evidence of its origin from a number of smaller vesicles. Thus in Figure 99 the fusion is still imperfect, portions of the vesicle walls still persisting as partitions. However, the fusion becomes more and more complete (Figs. 99–104), until, as shown in Figures 105 and 106 (Plate **7**), there is no evidence that the acrosome has arisen from a number of vesicles.

When the elongation of the cell begins, changes also occur within the nucleus preparatory to its condensation and lengthening. The first observable alteration affects the arrangement of the chromatin. We have seen that at the time of the migration of the centrosome from the cell membrane toward the nucleus, all, or nearly all, of the chromatin bodies collect upon the side of the nucleus nearest the centrosome. This aggregation of the chromatin persists until the axial filament has attained a

considerable length and the elongation of the cytosome is about to begin (Fig. 91). Then the chromatin bodies become scattered throughout the nucleus (Figs. 92, 93, 94). Next they undergo a process of dissolution by which all chromosomic boundaries seem to be broken down (Figs. 95, 96, 98, 99). The first stages in this are shown in Figures 95 and 96, where some of the chromosomes have already broken down and the chromatic material has been deposited in irregular masses upon the linin framework of the nucleus. This change continues to involve more and more of the chromatin until, at the stage represented in Figure 98, the only definite body of chromatin in the nucleus is the karyosphere.

Soon after this stage the chromatin begins to undergo a still more fundamental change. Already, in the granular condition (Figs. 97, 98), it seems gradually to become dissolved in the hyaloplasm of the nucleus. Stages in this transformation are shown in Figures 99–102. Most of the chromatin seems to become collected into one or more dense, irregular masses of granules at the centre of the nucleus, where it is surrounded by a clear layer of hyaloplasm (Fig. 99). The remainder of the chromatin occurs in the form of granules or small masses of granules irregularly disposed about the peripheral region of the nucleus (Figs. 99, 100). The nuclear sap at this place has not the same character as the more central karyolymph, but is very appreciably stained by Congo red (Fig. 99). It is evident that this difference in staining is due to the dissolution of the chromatin masses, for as these gradually disappear, more and more of the nucleus exhibits this reaction to the stain (Figs. 100, 102). As this process continues, the amount of chromatin staining in the ordinary manner becomes less and less, until at the stage of Figure 102 only a little remains. This stage may be taken as the period of greatest diffusion, for all of the nuclear substance, except the small quantity of unmodified chromatin and a small area immediately surrounding it, assumes a reddish gray color. Thus, while the chromatin which undergoes this chemical change loses its characteristic reaction toward stains, it still shows its presence in the nucleus by the deeper coloration of this entire structure.

Meantime other changes in the nucleus are taking place. Attention has been called to the fact that, up to the time when the elongation of the cell body is well under way, there is no continuous nuclear membrane. Shortly after the acrosome vesicles begin to appear, however, the nucleus becomes much smaller and a distinct membrane is formed (Figs. 96–99). It is highly probable that the nucleus takes an important part in the formation of the acrosome, for when the first indications

of this structure appear the nucleus is still without a definite membrane, and throughout the whole of the early development of the acrosome it decreases in size as the acrosome grows. It is possible that the material which acts as a solvent for the archoplasmic spherules is derived from the hyaloplasmic portion of the nucleus, rather than from the cytoplasm, as these vesicles are always very near to, if not actually in contact with, the nucleus (Figs. 95, 96, 98), and their contents show the same lack of stainability which characterizes the nuclear sap. However, it cannot be doubted that the archoplasm is the chief contributor in the formation of the acrosome.

With the change in the chemical nature of the chromatin (Fig. 99), the nucleus decreases considerably in size and at the same time undergoes a change of form (Figs. 98–100). From the almost spherical condition it passes at first into an irregular and then into a slightly elongated form (Figs. 96, 97). Afterwards the posterior face becomes more or less flattened, until in some cases (Fig. 98) the nucleus appears truncate. This condition is succeeded by one in which the whole nucleus becomes much more elongated (Fig. 102). The further changes in the nucleus will be described later.

Returning, now, to a further consideration of the axial filament during the elongation of the spermatid, the question naturally arises, where does the material for its growth come from? It has been shown that in all probability the material for the formation of the proximal portion of this structure is obtained from the archoplasm, for at first it is completely enveloped in the mass of this substance which likewise surrounds the centrosome. Later, however, when it has outgrown this visible accumulation of archoplasm, it probably grows at the expense of that portion which is still disseminated through the cytoplasm. But as the axial filament continues to increase in length, the archoplasm in solution in the cytoplasm becomes diminished, as is indicated by the increasing transparency of the inter-reticular areas (Figs. 92–94).

About this time, which coincides with the beginning of the elongation of the cell and with the detachment of parts of the archoplasm to form the fundaments of the acrosome, other portions of the archoplasm break off from the mass surrounding the centrosome and migrate in an opposite direction along the axial filament. Various stages in this process are shown in Figures 92–96. In Figure 92 one of these archoplasmic masses is just leaving the central mass and beginning its migration along the filament. In Figure 93 two such bodies have broken off and a third is in process of forming. In Figure 94 several of these have

proceeded a considerable distance in their migration, while several more are already definitely formed. In Figures 95 and 96 all the archoplasm except a very small portion, which remains permanently in this region, has migrated along the axial filament.

Recalling the phenomena connected with the origin of the axial filament and the early stages in its growth, I think but one conclusion can be drawn regarding the significance of the migration of these archoplasmic bodies. They contribute to the further growth of the axial fibre. At the stage represented in Figure 96 these small deeply stained granular masses may be seen at various points along the course of the filament and always close to it. In later stages they are also found, but they are then much less definite in outline and less deeply stained. Apparently their substance is gradually broken down during this period of the elongation of the cell and the continued growth of the axial filament in length and thickness.

However, there are other phenomena which might be construed as indicating a different origin of the filament. In Figures 95 and 96 are shown at various places in the cytoplasm fine short granular or monilated fibres which in many ways resemble the beginnings of the axial filament. Like it they are stained black by the haematoxylin-Congo-red method. As a general thing they lie in the reticulum of the cytoplasm and often appear merely as thickenings of it. From this it might be argued that the axial filament itself is derived directly from the cytoplasm by the mere condensation and fusion of parts of the reticulum. However, there are frequently structures of this kind which have no connection with the reticulum whatever, but lie free in vacuoles of hyaloplasm. Figure 101 shows a striking example of this condition, but similar conditions are also to be observed in Figures 95 and 96.

From these phenomena and those already described in connection with the earlier stages in the formation of the filament, it is very improbable, I think, that the axial filament arises directly from the cytoplasmic reticulum, although it is possible that the network may undergo a chemical change similar to that experienced by the archoplasm and thus may afford some of the material for the formation of the axial filament.

The typical arrangement of the centrosomes shortly after the beginning of the axial filament — one on each side of this structure and one forming an enlargement at its proximal end — continues to exist until the elongation of the cell body is well under way (Figs. 93, 94). After this stage the two lateral centrosomes are invisible for a considerable time;

in fact, they are not seen again until the cell in its metamorphosis has reached the stage represented in Figure 102 (Plate 7). However, the centrosome at the end of the filament persists and may be seen at almost every stage.

From the appearances seen in Figure 102 and in later stages, it cannot be doubted that the two lateral centrosomes, at the time of their disappearance, enter the nucleus, which at this stage (Figs. 95, 96) is devoid of a membrane, and for a time become lost to view among the masses of chromatin. They are not seen again until the chemical nature of the chromatin has become so altered that it no longer assumes the black color (Fig. 102). At this and all succeeding stages the two lateral centrosomes are seen to be contained within the nuclear membrane, and are at all stages distinguishable, although for obvious reasons they are not seen in all cells.

It may perhaps be of significance that at the time the centrosomes enter the nucleus (Figs. 95, 96) the chromatin is in the granular form and exhibits its characteristic reaction to stains, whereas immediately afterward it begins to undergo a transformation by which all of its morphological and chemical characteristics are altered.

The lateral centrosomes when they again become visible within the nucleus (Fig. 102) are at first small, inconspicuous, deeply staining bodies, which retain the position in relation to the axial filament that was characteristic of them during preceding stages. Each one is now indubitably connected with the central centrosome, or end knob, by means of a conspicuous fibre, which follows the outline of the nuclear membrane. This condition is much better shown in Figure 103, which is a drawing of one of the small spermatids at a slightly later stage.

As the nucleus continues its transformation it assumes the shape shown in Figures 104 to 108. In all longitudinal sections it appears dart-shaped. The contents of the nucleus at this time are beginning to regain their affinity for stains, and small masses of granular chromatin are again appearing (Figs. 104, 105). This change continues to become more and more marked, until at the stage represented by Figure 106 the nucleus as a whole assumes a very deep black stain. However, in all properly stained preparations, there are certain areas which are not so deeply colored; these give rise to such appearances as are represented in Figures 106–108. Thus, at the anterior end of the dart-shaped head of the young spermatozoön there is an area of varying extent which shows a paler coloration. This is because the interior part of the head here remains unstained, while a thin deeply stained layer upon the out-

side can be distinctly seen (Figs. 107, 108). It appears as though there were a vacuole of less deeply stained material in this region. Occasionally smaller vacuoles are present in all parts of the head (Figure 106). Furthermore in all cells in which the decolorizing process has been carried sufficiently far, a lighter area is seen at the base of the head (Figs. 107, 108).

This, from one point of view, presents the appearance shown in Figure 107, whereas, viewed in another direction, it appears as in Figure 108. Here the two lateral centrosomes and the end knob are very plainly visible. In fact, they more closely resemble typical centrosomes than do the same bodies in the stages immediately preceding (Figures 104, 105). There can be little doubt of their identity, even at stages when numerous similarly staining chromatin granules are present in other parts of the nucleus. In Figure 108 there can be no doubt whatever as to the nature of these bodies, for none of the chromatin of the nucleus takes on this form. Furthermore, each of the lateral centrosomes is surrounded by a fairly distinct clear area and is connected with the centrosome at the base of the filament by a distinct fibre. When viewed at right angles to this, the lateral centrosomes are usually not visible (Fig. 107), but when they can be seen they lie at such different levels that both do not appear at one focus.

The head of the young spermatozoön continues to elongate and soon assumes the appearance represented in Figure 109. By careful decolorizing, all of the chromatin exhibits a paler grayish color, while the centrosomes are readily distinguishable both by reason of their position and by their stronger retention of the stain. At this stage the base of the nucleus forms a plane perpendicular to the long axis of the nucleus, and the two lateral centrosomes are so closely apposed to the membrane that each is flattened out into a hemispherical body. These two bodies lie side by side, quite close together, but each is distinct from the other (Fig. 109). The connection between intranuclear centrosomes and the one at the base of the axial filament is very evident, and presents the appearance of a number of fibres forming a sort of membrane rather than that of a single thread.

At this stage (Fig. 109) a peculiar cuff-like structure, apparently attached to the posterior margin of the head, is often to be observed. What its function may be I cannot say, for I have been able to find no such structure in the more advanced stages. Its origin and history are of interest, as at an earlier stage it is similar to structures described in other myriapods (Tönniges :**02**) and in the guinea pig (Meves, '**99**).

At the stage when the lateral centrosomes enter the nucleus (Figs. 95, 96), there remains closely apposed to the nucleus a small hemispherical mass of archoplasm. This persists and retains its form for a considerable time (Fig. 98), but by the time the elongation of the nucleus has become pronounced, this archoplasm has been converted into a number of distinct fibres, which in general extend parallel to the axial filament (Fig. 100). When these first arise they are quite distinct threads irregularly disposed in the region immediately posterior to the nucleus and stained by the Congo red. Soon, however, they collect toward the periphery and apparently become attached to the nuclear membrane, forming in longitudinal sections a line continuous with the lateral outline of the nucleus and extending backward a distance equal to half the length of the nucleus (Figs. 102–104). The fibres are now coarser, due to the fusing of several into one, and stain a reddish gray. This fusion continues as the diameter of the nucleus becomes less, until, as shown in Figures 105, 106, and 109, the fibres have all united to form the cuff-shaped structure already described. The region enclosed by this often stains much less deeply than the rest of the cytoplasm (Figs. 104, 105, 109). This is, I believe, due to the aggregation of the fibres at the periphery of the region in the manner described. The cytoplasm does not immediately fill the space thus left, and therefore it is occupied by the more transparent hyaloplasm only.

We have seen that, at the conclusion of the fusing of the small vesicles to form the acrosome, this is a large irregular pyriform or club-shaped vesicle often exceeding the nucleus in bulk (Figs. 102–104). The distal end is usually more or less enlarged, so that the irregular pear shape is the more common. In the earlier stages of its formation (Figs. 99–103) its proximal constricted end is in contact with the nuclear membrane. Occasionally, possibly by the action of the fixing reagent, the membrane, which is quite prominent, is ruptured and the vesicle collapses as shown in Figure 100.

Shortly after the acrosome vesicle has assumed its characteristic shape (Fig. 103), a second much smaller vesicle appears at its basal end where it comes in contact with the apex of the nucleus (Fig. 104). This for a time becomes larger and larger (Figs. 105, 106, 109), and then gradually diminishes in size (Figs. 110, 111), until in the mature spermatozoön no evidence of such a structure remains (Fig. 121).

When the chromatin in the nucleus again begins to regain its accustomed affinity for stains (Figs. 104, 105), the acrosome also undergoes a change by which its staining reaction is altered. Its volume is very

much decreased, and its form becomes more definite and regular (Figs. 105, 106). I think it probable that the increased intensity of the staining is due to the condensation of the acrosome material rather than to the absorption of material from the nucleus. It is the peripheral portion only that is stained, the material within the cavity of the vesicle remaining entirely transparent.

The acrosome retains the pear or club shape for a considerable time (Figs. 105, 106), but when the nucleus begins its second period of lengthening (Fig. 109) the acrosome also undergoes a similar change. The first stages in this process are rather peculiar. One would naturally expect the vesicle merely to elongate through its whole length, but such is not the case. A protuberance appears upon one side of the acrosome near the anterior end; this gradually extends farther and farther forward, giving it the peculiar appearance shown in Figure 109. As the process continues to elongate, the contour becomes more regular and the acrosome soon assumes the form of a thick filament, which tapers slightly toward the anterior end, but does not terminate in a sharp point (Figs. 110, 111). From this stage the elongation continues until, in the mature spermatozoön, the acrosome is a slender filament sometimes nearly 100 micra in length and correspondingly slender, its greatest diameter being rather less than 2 micra. The proximal end is blunt or rounded and is connected directly with the anterior end of the head (Fig. 121). The distal or anterior part of the acrosome tapers very gradually and ends in a sharp point (Fig. 121). No barbules, such as are of frequent occurrence in other animals, have been observed.

After the stage represented in Figure 109 the head of the young spermatozoön elongates rapidly and soon has the form shown in Figure 110. At this time numerous small vacuoles are in some cases to be seen in all parts of the head (Fig. 111), while in other cells there are one or two large vacuoles, which occupy nearly the entire central portion (Fig. 112). Cross-sections of spermatozoa in this condition are shown in Figure 113, where the diameter of the chromatic sheath is about one fourth that of the entire head. This vacuolation continues to become more marked, and as the vacuoles unite a larger and larger one is formed, which in the mature spermatozoön occupies the entire central region and extends the whole length of the head (Figs. 116–121).

As the elongation continues, the anterior end of the head becomes tapering (Fig. 114). At about the same time the first indications of a spiral condition are to be observed. This condition is first apparent near the base, and in longitudinal section it presents the appearance of

tooth-like projections arising from opposite sides of the head, those on one side alternating with those on the other. This appearance is due to the presence of a spiral ridge upon the surface of the head.

In later stages this spiral continues to become more and more marked, and the head soon appears as represented in Figures 116–118. Here the more transparent inner portion is plainly shown, all of the chromatin being arranged at the periphery. A careful study of this stage shows the character of the spiral thickening of the head. In Figure 115 are represented a transverse (*a*) and a longitudinal (*b*) section and a surface view (*c*) of the head at this time. By studying these figures it is at once seen that the spiral represents not only a ridge, but that the crest of the ridge consists of a much thicker deposit of chromatic material than exists elsewhere on the head. This causes the spiral to be even more conspicuous than it otherwise would be. This is very well shown in a study of cross sections of the head (Fig. 115 *a*). In studying the sections this denser chromatic portion lying upon one side of the head may be seen to shift its position regularly as the microscope is focussed up and down, so that it makes an entire circuit of the cross section. In longitudinal section (Fig. 115 *b*) the appearance leads to the same conclusion, for here we get an oblique section of the chromatic thickening. Figure 115 *c* is a reconstruction based on the cross and the longitudinal sections, but the same appearance may be actually seen occasionally where the staining is of exactly the right intensity.

At the stage shown in Figure 116 the head of the spermatozoön is sometimes compressed in the plane of the two centrosomes, thus forming a ribbon-like structure. This I consider to be due to the loss, at the moment of fixation, of the fluid contained in the central region. It is of great assistance in studying the centrosomes at the base of the head and in obtaining a clear idea of the peripheral disposition of the chromatin. Figures 117 and 118 represent portions of spermatozoa which have suffered this loss of material and have assumed a ribbon-like form. In both, the two lateral centrosomes are unmistakably evident, as they lie in close apposition at the base of the head. These flattened heads also very often afford an excellent optical section of the entire structure, showing the peripheral band of chromatin upon each side of a central unstained region. In Figure 118 this ribbon-like structure is placed with the flat sides parallel to the plane of the section. In Figure 117 the "ribbon" has been twisted and thus affords optical sections from the edge as well as the side.

From this time forward, the transformation of the spermatozoön con-

sists entirely in its further elongation and in the continued development of the spiral. Thus in the mature spermatozoön (Figs. 120, 121) the spiral is so pronounced that a straight line drawn through the centre of the head would barely fall within the limits of this structure throughout its entire course. The unstained central portion of the head is present, just as in preceding stages, and may be observed both in cross and longitudinal sections, and is occasionally shown even in surface views (Fig. 120).

In these later stages of metamorphosis the centrosomes are still readily to be demonstrated. The two lateral ones lie in the position that characterized them at previous stages, i. e. side by side at the base of the head and enclosed in the nuclear membrane of the head. Extending backward from these two centrosomes is a triangular structure, evidently made up of fine fibres, which joins the lateral centrosomes with the end of the axial filament (Figs. 116–120). The triangular region thus enclosed stains much less deeply than the axial filament proper. In some cases there seem to be two very distinct fibres at the border of the triangle (Fig. 119), but such appearances are rare. This triangular region varies in its proportions, being usually long, with the acute angle directed toward the axial filament (Figs. 117, 118, 120), while occasionally it may be more nearly equilateral (Figs. 116, 119). When the spermatozoön is viewed parallel to the plane of the three centrosomes (Fig. 120), no evidence of this triangular region is to be observed, showing beyond a doubt that the triangle represents a membrane or more probably a number of fibres lying in one plane and spreading out from the base of the axial filament like the ribs of a fan, to join the lateral centrosomes at the base of the head.

It has been noted that at earlier stages there is always an enlargement at the basal end of the axial filament. This structure, which is derived directly from the original spermatid centrosome, retains its close connection with the axial filament throughout all the transformation stages, and may often be seen in the fully developed spermatozoön as a very slight enlargement at the apex of the triangle described above (Fig. 116). In many spermatozoa, however, this element cannot be observed, and it is always very inconspicuous. From its long-continued persistence throughout all the transformations, and its presence in many of the mature spermatozoa, I believe there can be no doubt of its importance. In position and origin it corresponds to the end knob in the spermatozoa of many animals.

One of the most remarkable facts concerning the mature spermatozoa

is the extremely elongated form of the head and the remarkably large acrosome. As I have already stated, there is much variation in the size of the cells at all stages after the growth period of the spermatocytes, and this is very noticeable in the spermatozoa as well as at other times. I have represented in Figure 121 what may be regarded as the greatest size. Here the actual length of head and acrosome is 260 micra, and of this length the nuclear portion represents 170 micra, while the width of the head is only about ½ micron. It would be interesting to know whether there is any condition existing in the egg of Scolopendra which would require a spermatozoön of such attenuated form. The smaller spermatozoa are of about the same diameter as the large ones, but are much shorter, the heads of some being only about 75 micra in length. It is noticeable that these smaller heads stain more strongly than the larger ones, probably owing to a greater concentration of the chromatin.

Throughout all the later processes of transformation the lengthening of the cell has proceeded at the same rate as that of the contained structures, and in the fully developed element the entire spermatozoön, — acrosome, head, and flagellum — is surrounded by a rather thick layer of cytoplasm, bounded by a definite membrane (Figs. 120, 121). This is perhaps best shown in cross sections. In those through the head region this layer of cytoplasm occupies at least two thirds of the diameter, which seems to be about the same in all regions of the cell. In the tail region (Fig. 113) the axial filament lies near the centre of the cross section and is surrounded by a layer of dense cytoplasm.

This study of spermatid changes has been made exclusively upon sectioned material; while this is undoubtedly very favorable for studying the details of the metamorphosis, it is not so valuable for the study of the general structure of the mature sperm cells as preparations of dissociated material would have been. It is of course practically impossible to obtain a longitudinal section of the entire spermatozoön, owing to its great length and to the fact that it is usually distorted when killed. For this reason I am unable to give the general topography of the spermatozoön or of the cells in the later stages. I cannot be absolutely certain of the relative length of head and flagellum, but can say with certainty that the latter structure is several times as long as head and acrosome together. Thus the mature spermatozoa derived from the larger spermatocytes must reach a length of nearly one millimetre, for the head region is one fourth this length by actual measurement.

In Figure 121, which represents a mature spermatozoön as found in the central region of the follicle, there is quite a thick envelope of cyto-

plasm surrounding the head region. Whether this is true of the spermatozoön after it has been set free in the vas deferens, or whether this envelope is reduced to a thinner layer, I cannot state, but the latter would seem to be necessary for its active swimming and for its penetration of the egg at the time of fertilization. However, in the latest stages occurring in the follicles of the testes this sheath of cytoplasm is present.

## IV. Discussion of Literature.

### 1. Nuclear Structures.

#### A. *The Karyosphere.*

I shall first discuss somewhat briefly the structures which in Protozoa correspond to the karyosphere, and later the nucleolus-like structures in the cells of Metazoa which present marked points of similarity to this body.

*In Protozoa.* — Calkins (:01, pp. 245–278), from his own work and that of numerous other investigators upon the cell structure of the Protozoa, has arrived at conclusions concerning nucleolus-like structures in this group which are of great importance not only in considering unicellular animals, but also in studying the analogous structures in the Metazoa. With regard to the true nucleolus Calkins has the following to say (p. 253): "A distinct plasmosome or true nucleolus comparable to the analogous structure in Metazoa apparently exists in no case save possibly in Actinosphaerium, and even here it is limited to a passing phase during mitosis (Hertwig, '98). It is probable that the structures which have been almost universally but erroneously called nucleoli, do not belong at all to this category of nuclear elements, but represent either the functional chromatin which is aggregated into a central mass (karyosome) during the quiescent or vegetative period of cell life, or the intranuclear division centre."

The lower types of nuclei often show considerable resemblance to structures in the metazoan cell which have been called nucleoli or karyosomes. Within the more differentiated nuclei of Protozoa are often found masses of chromatin and achromatin which are nearly identical with some of the "nucleoli" found in Metazoa, and at the same time are strictly analogous to the nuclei of less modified Protozoa. The nuclei of primitive Protozoa more nearly resemble in structure and general behavior the *karyosomes* of many Metazoa and higher Protozoa than they do the nuclei of these more highly differentiated cells.

In Protozoa (Calkins, :**01**, p. 253) "Five types of nuclei, based upon the disposition of the chromatin, can be distinguished. Of these the most primitive is, (1) the solid sphere, or karyosome (Binnenkörper, Rhumbler), which has neither linin reticulum nor membrane (e. g. *Calcituba*). An advance is shown in (2) nuclei having one such karyosome surrounded by karyolymph, the whole enclosed within a membrane (vesicular nuclei, Gruber, '84); while still higher types are (3) nuclei with several karyosomes (two to thirteen or fourteen), with membrane, karyolymph, and with or without a nuclear reticulum (e. g. *Noctiluca*); (4) nuclei with a large number of smaller masses of chromatin enclosed in a definite membrane with or without a linin reticulum (e. g. *Amoeba proteus*); (5) nuclei consisting of granules of chromatin unconfined by a nuclear membrane and spread over the entire cell (distributed nucleus) or aggregated about a central body ("intermediate" type of Calkins, '98, e. g. *Tetramitus*)."

Thus the more simple nuclei, the first type, are but single homogeneous masses of chromatin, i. e. karyosomes. Those of the second type show some advance. They contain achromatin as well as chromatin and are surrounded by a definite membrane. "The chief interest (p. 254) of these nuclei, however, centres in the chromatin mass, the 'karyosome' of Labbé ('96), which, as in similar nuclei among the *Sarcodina*, has been described under several names." It often appears homogeneous, but is not really so. "The cortical portion consists of chromatin with an exceedingly fine alveolar structure." The history of this karyosome is strikingly similar to that of the isolated chromatin mass in Calcituba (1st type). A higher kind of this type is that found in Actinosphaerium (Hertwig, '**99**). In this genus "the karyosome in nuclei of ordinary vegetative forms is distinctly granular, the chromatin granules being grouped in a variety of ways." Hertwig says: "Das Kernnetz . . . enthält sicher kein Chromatin. . . . Ich muss an der früher von mir gegebenen Schilderung fest halten, das alles Chromatin in dem grossen Körper abgelagert ist den ich früher 'Nucleolus' genannt habe." This "Chromatinkörper" is usually colored deeply by the chromatin stains, and in the majority of cases seems to be of a granular consistency, although at times it appears homogeneous. In many of Hertwig's figures ('**99**, Taf. II. Fig. 8, 10, *et al.*) it is strikingly like the karyosphere of Scolopendra in appearance, while in others it differs considerably. The "Chromatinkörper" consists of two substances, "(1) das Chromatin, (2) das Material, welches die ächter chromatinfreien Nucleoli der Gewebszellen bildet, für welches Carnoy ('98) und

Zacharias ('85, '87) den Ausdruck, Plastin gebraucht haben." During the formation of the first polar cell, all the chromatin being withdrawn from this structure, a true plasmosome nucleolus is produced. However, upon the reconstruction of the nucleus the chromatin is again deposited in this body, and it resumes its wonted characteristics. This structure corresponds to the "nucléoles mixtes" of Carnoy ('85). In mitosis it breaks up into granules, which arrange themselves in parallel lines in the nucleus, resembling closely the chromatin segments of metazoan cells. In the next higher type, represented by Noctiluca, the chromatin is in the form of a number of karyosomes (generally 10–12) which, during the prophase, behave in a manner similar to the single one in Actinosphaerium; but there is no residue of plastin. The bodies found in the other types of nuclei do not appear to bear such a close relation to the structure in question and therefore need not be discussed here.

From these results I believe we may conclude that the lower type of nuclei found in the Protozoa is very similar in structure to the karyosome or karyosomes found in the more differentiated nucleus of this group and in the nucleus of many Metazoa. Indeed, they seem to be more nearly analogous to karyosomes than to the higher type of nuclei. The karyosomes found in some of the higher types of nuclei among Protozoa are not homogeneous masses of chromatin, but also contain, besides this substance, linin. The linin often forms a reticulum, upon which the chromatin is deposited in the form of granules. This arrangement is similar to that found in many of the nuclei in Metazoa, and gives rise to a structure which is similar to the chromatin reticulum of more differentiated nuclei.

It is, however, in appearance still more strikingly like the spireme structure of the karyosphere. That it is different from this, however, in some respects, is shown by comparing the subsequent behavior of the two structures; but this difference is one that would be expected when we take into consideration the difference in source. The chromatin elements are much more firmly established in the higher animals, and hence it is to be expected that when the karyosphere breaks down, the resulting fragments will be distinct chromosomes. In Protozoa the conditions are different. The chromosomes are not such definite structures, and hence when the karyosome disintegrates it gives rise to a large number of granules, which later aggregate into chromosome-like masses. However, I believe the relationship is sufficiently close to warrant our placing in the same general category the solid chromatin nuclei of some Sporozoa

and Rhizopoda, the karyosomes of the nuclei of higher Protozoa, and the karyosomes and karyospheres[1] found in the nuclei of Metazoa.

*In Metazoa.* — Nucleolus-like bodies other than true plasmosomes have been reported in the cells of a vast number of Metazoa. Structures apparently composed of pure chromatin (karyosomes) are of very frequent occurrence in tissue cells, and are especially characteristic of the large ganglion cells of nervous tissue. In germ cells they occur much more frequently in the female element than in the male. (The accessory chromosome in the male cells must be included in a different class of bodies, as it is a distinct element, a chromosome, as distinguished from karyosomes, which are not such definite structures.) Indeed, in the great majority of immature eggs the growth period preceding maturation is as a general thing characterized by the appearance within the germinative vesicle of nuclear bodies, some of which are plasmosomes, while many are plainly composed either entirely or in part of chromatin. In many cases these chromatin bodies have been described as taking no part in the subsequent activities of the nuclear structures. The chromosomes are not derived from them, and the karyosome upon the disintegration of the nuclear membrane is either set free in the cytoplasm, where it degenerates (Wheeler, '95; Mead, '98; Griffin, '99, and others), or previous to the maturation divisions is extruded from the germinative vesicle and gives rise to yolk material (Balbiani, '93, *et al.*).

In many eggs in which the "nucleolus" behaves in this manner, it is said to be derived by a chemical transformation, not from the chromatin reticulum of the nucleus, but from other sources. However, in some cases the authors assert that these bodies are formed from material derived from the functional chromatin of the cell. The chromatin reticulum within the nucleus, so they affirm, breaks down and later aggregates to form a large karyosome. Yet this is later cast out into the cytoplasm and no longer functions as chromatin. I believe it is very improbable that chromatin which has once been a part of the chromosomes should be eliminated in this manner without an accurate division such as occurs at the time of the formation of the polar cells. Such reported cases

[1] In the use of the term "karyosome" I limit it to structures found within the nucleus which are composed exclusively of chromatin but are not discrete chromosomes. The karyosphere is much more complex, for it contains chromatin (in granular, reticular, or spireme form), karyoplasm in the form of linin, and karyolymph; it may also contain nucleolar material. It is, in fact, a miniature nucleus. True nucleoli or plasmosomes come under an entirely different category, for karyosome and nucleolus have no real analogy with each other, the only points of similarity being their intranuclear position and approximately spherical shape.

should be re-examined, for if established this would throw great discredit upon the theory of the individuality of the chromosomes.

While many investigators seem to favor the view that the large "chromatin nucleoli" of germ cells have nothing to do with the formation of the chromosomes, several assert that the chromosomes are derived directly from their substance, and in some cases offer excellent evidence to establish their conclusions. Thus we see that there are several important matters to be considered in discussing these chromatin-bearing nucleoli. Among these are (1) their origin, (2) their structure, and (3) their function and behavior during mitotic division.

In considering the phylogenetic origin of the karyosphere and similar chromatin-bearing bodies it has been seen that they are probably genetically connected with the karyosome composing the lower type of nuclei in Protozoa and the similar structures (karyosome, Calkins; Binnenkörper, Rhumbler; Chromatinkörper, R. Hertwig) found in the higher types of nuclei in these unicellular animals. We shall now consider their ontogenetic origin in metazoan cells. According to the views of different authors they are either (1) of achromatic origin, produced by a chemical change in the achromatin of the cell, or (2) of chromatic origin, obtained from the chromosomes or chromatin reticulum of the nucleus.

Concerning the bodies of achromatic origin, I shall say nothing here, except that I believe it very improbable that chromatin which arises in this manner — i. e. having no connection with the true chromatin elements of the cell — should have any important significance in the succeeding mitoses. This may account for the fact that in the maturation division of many cells "nucleoli" staining like chromatin are cast out into the cytoplasm, where they subsequently degenerate. Such a behavior has been reported in a large number of metazoan cells (Häcker, '93, in Aequorea; Wheeler, '95, in Myzostoma; Mead, '98, in Chaetopterus; Griffin, '99, in Thalassema, Zirphaea; and others). Such is probably also the origin of the chromatic or nucleolar material which is discharged from the resting nuclei into the cytoplasm, as reported by Balbiani, '93 (Geophilus, etc.), Jordan, '93 (newt), Calkins, '95 (Lumbricus), and others. It is very improbable indeed that such an extrusion of nuclear substance involves material derived directly from functional chromatin.

Many investigators, however, assert the second alternative — i. e. that the "chromatin nucleolus" is derived from the chromatin reticulum of the nucleus. Among this number may be mentioned the following: Blochmann, '82 (Neritina); Van Beneden, '83 (Ascaris); Van Bambeke '85 (general); Carnoy, '85 (arthropods); Rabl, '85 (Salamandra);

Schultze, '87 (Rana and Triton); Davidoff, '89 (Distaplia); Hermann '89 (Mans); Macallum, '91 (Rana and Necturus); Schneider, '91 (Echinodermata); Fick, '93 (Axolotl); Holl, '93 (Mus); Jordan, '93 (newt); Mertens, '94 (Pica); Metzner, '94 (Salamandra); Macallum '95 (Necturus, and plants, Erythronnium and Spirogyra); Sobotta, '95 (Mus); Hertwig, '96 (Poisoned eggs of Echinodermata); Carnoy et Lebrun, '97 (Amphibia); Eisen, :00 (Batrachoseps); Wilson, :01 (chemically fertilized eggs of Toxopneustes); and Blackman, :01, :03 (Scolopendra). In many of these cases the process was followed in considerable detail, while in others the conclusions are not so well founded. In a considerable number of instances the karyosphere contains all of the chromatin of the cell, as is shown in the prophase of mitosis, where the chromosomes are often plainly formed from and employ all of its substance. Such a case is found in the egg of Neritina (Blochmann, '82). This author says: "Dass die Elemente der Kernplatte aus Theilstücken des Nucleolus entstehen, kann bei unserem Objekt keinen Zweifel unterliegen, da ich alle Uebergangszustände vom unversehrten Nucleolus bis zur ausgebildeten Kernplatte beobachtet habe." Van Beneden ('83), in working upon the ovum of Ascaris megalocephala, finds that all the chromatin is aggregated into one mass, which he calls the "corpuscule germinatif." Kultschitzky ('88) obtained similar results upon A. marginata. However, the observations of other investigators have led to quite different conclusions and thus cast doubt upon the earlier work with Ascaris.

Carnoy's ('85, p. 207) results upon the arthropods I shall consider more in detail, as some of his conclusions are based upon a study of several genera of Chilopoda. He divides the nucleoli into four classes as follows: "(a) Les *nucléoles nucléiniens;* sphérules de nucléine amorphe, ou ramassée en peloton serré. Ils se colorent par le vert de méthyle et se dissolvent dans l'acide chlorhydrique concentré. (b) Les *nucléoles plasmatiques:* masses albuminoides renfermant de la plastine. Ils demeurent incolores sous l'action du vert de méthyle, et ils résistent à l'action des dissolvants de la nucléine. Ces nucléoles se rencontrent assez rarement dans les cellules testiculaires des arthropodes; celles des scolopendres en possèdent un bien marqué. (c) Les *nucléoles mixtes*, qui sont constitués par la réunion des deux espèces précédentes en un corps unique, où chacune se maintient cependant sous une forme figurée. (d) Les *nucléoles-noyaux*, ou noyaux en miniature, renfermant par conséquent tous les éléments d'un noyaux véritable; membrane, portion plasmatique et portion nucléinienne. Le type de ces nucléoles nous est offert par les Lithobius."

The classes (a), (c), and (d) are similar in that they all contain chromatin, but differ in the manner in which this chromatin is arranged. Of these three classes the last (d) is the one which most resembles the karyosphere as observed by myself in Scolopendra heros. This structure in Lithobius is in all respects like a miniature nucleus. It contains all of the chromatin of the cell in the form of granules deposited upon a reticulum of linin fibres, and is surrounded by a definite membrane. This membrane I have never been able to demonstrate in Scolopendra, and indeed I do not believe that it exists there, although it may well be present in Lithobius. The nucleolus gives rise in the first maturation division to the chromatin thread, from which the chromosomes are derived. After the completion of the first division it is said to be again reformed. On the reconstruction of the daughter nuclei (p. 301), "On peut en effet y distinguer deux étapes; (a) la formation du nucléole, (b) la formation du noyau proprement dit. La nucléole se reconstitue le premier, et a la façon d'un noyau ordinaire." From my own observations upon Scolopendra and more superficial study of Geophilus, Scutigera, and Lithobius, I am convinced that no such process ever occurs. Carnoy's Figures 210 and 216, which he has interpreted as the telophase of the first maturation division, are indeed drawings of cells in the vesicle stage. Carnoy also observed this "nucléole noyau" in two other genera of Chilopoda, Scutigera and Geophilus, where in structure and behavior it is very similar to that in Lithobius. But in Scolopendra dalmatica the "nucleolus" is found to belong to an entirely different class. Concerning this body he speaks as follows (p. 302): "Le boyau y est uniformément distribué, et le nucléole . . . n'est pas un nucléole-noyau, mais un *nucléole plasmatique*. Sa constitution est donc celle d'un noyau ordinaire." Later he says: "Le début de la caryocinèse s'annonce par plusieurs phénomènes concomitants: la scission du boyau, la fusion du nucléole, l'apparition des premiers rudiments du fuseau et enfin la naissance des asters." Then, after describing the formation of the chromosomes, he adds: "En même temps que le boyau se segmente, le nucléole se liquéfie pour enrichir le caryoplasma; c'est en vain que nous l'avons recherché dans les phases subséquentes."

Thus the facts of the case in S. dalmatica seem to be these. One of the first phenomena of mitosis is the disintegration of the nucleolus. This is accompanied by the formation of the chromosomes. Later, when the chromosomes are completely formed, the "nucleolus" is no longer to be seen. These observations as far as they go entirely agree with mine on S. heros, and it seems possible that further study might

lead to conclusions like my own. In well-fixed and well-stained preparations of S. heros no other conclusions than those I have presented seem possible, and I believe that the same is true of S. dalmatica. The apparent disappearance of this element in the late prophase can also be very easily explained. The "nucleolus," bereft of the material going to form the ordinary chromosomes, is now identical with its condition before the vesicle stage, i. e. it is again merely the accessory chromosome, and is so closely similar to the other chromosomes of this stage that it can be distinguished from them only in very favorable material.

By the great majority of investigators of amphibian ova it is agreed that at certain periods in the development of the egg the chromatin is either entirely — Schultze, '87 (Rana and Triton); Carnoy et Lebrun, '97, '98, :00 (Amphibia) — or at least partially — Macallum, '91 (Rana and Necturns); Jordan, '93 (newt)[1]; Fick, '93 (Axolotl) — collected in the form of a number of nucleolus-like bodies. The observations of several other investigators — Metzner, '94 (Salamandra); Eisen, :00 (Batrachoseps) — upon spermatogenesis in Amphibia would indicate that similar phenomena sometimes occur in the male germ cell as well; and the works of Rabl, '85 (Salamandra), and Lavdowsky, '94 (Amphibia) would indicate that the somatic cells of larvae behave in a similar manner. From these observations there can be no doubt that in numerous cases the chromatin reticulum of Amphibian cells is wholly or partly broken down and becomes aggregated into a large number of pseudo-nucleoli.

A similar process also undoubtedly occurs in the germ cells of Mus according to the observations of Hermann ('89), Holl ('93), and Sobotta ('95). Hermann, working upon mouse material, reports nucleoli composed of chromatin as present in the cells at various stages of spermatogenesis. In the spermatid there are at first several, but later these fuse to form a single large one. Holl reports that in the germinative vesicle of the mouse ovum there is a large nucleolus composed chiefly of chromatin, from the substance of which the chromosomes are derived during the prophase of the first maturation division. Sobotta states that during fertilization stages the pronuclei, both male and female, contain generally one but often several large nucleoli, which under the highest powers appear to be homogeneous masses of pure chromatin. "Der ganze übrige Kern repräsentirt nur ein achromatisches Kerngerüst."

[1] The nucleolus in this material is derived from the reticulum of the nucleus, but at the same time is said to take no part in the subsequent formation of the chromosomes.

Later the "Nucleolen" break down and contribute their substance to the formation of the chromosomes. "Die Nucleolen fangen an sich heller zu färben, während sich auf den achromatischen Gerüststrängen Chromatintheile in feinster, unregelmässige Form vertheilen." They become vacuolated and finally appear in section "als etwas unregelmässige chromatische Ringe." At this time "Das Chromatin ist in allerfeinsten Flöcken auf dem ganzen achromatischen Kerngerüst vertheilt." The chromosomes are later formed from these irregular granular masses.

In the egg of one of the lower chordates (Distaplia), Davidoff ('89) has observed that the large nucleolus increases in size by the addition of the greater part of the nuclear reticulum, and that this material is later used in the formation of the chromosomes of the polar spindle.

Schneider ('91) asserts that in the eggs of Echinoderms the nucleoli are only reserve masses of metamorphosed chromatin. That this is true in some cases at least is shown by the recent observations of Wilson (:01[a]) upon the eggs of Toxopneustes artificially fertilized with a solution of magnesium chloride. He finds that in one series of the eggs thus treated the chromosomes taking part in mitosis are obtained by the breaking down of the large deeply staining nucleolus. "Its contour becomes irregular and its texture loose. A little later it assumes a spongy appearance and short irregular processes are extended from its periphery. Enlarging still more, it now gives almost the appearance of a close, broken spireme, from the ends of which chromatin threads here and there project." In the other series the chromatin is retained in the form of a reticulum, as in the normal eggs, and the chromosomes are derived from this reticulum. In attempting to explain this variation he says: "I think that in one case the chromatin is gradually drawn from the linin network through which it is originally distributed. In the second case such a withdrawal does not take place, though the achromatic nucleolar material accumulates as before." These conclusions of Wilson confirm the earlier observations of Hertwig ('96), who obtained similar results upon Echinoderm eggs treated with strychnine.

In Limnaea Linville (:00) also reports observations which he thinks indicate a close connection between the nucleoli or karyosomes and the chromatin elements. He however has made no detailed study of the chromatic structures, and therefore hesitates to assert definitely that the chromosomes are of nucleolar origin.

From the preceding brief review it will be seen that in Protozoa the aggregation of the chromatin into karyosomes or more complex karyospheres is of general occurrence, while the presence of the spireme or

chromatin reticulum in resting stages is exceptional, being found in only a very few of the most highly developed nuclei. In Metazoa these "chromatin nucleoli," while not of such general distribution, are present in a large number of both somatic and germ cells and in some groups seem to be of nearly universal occurrence. In the Chordata they are found in a number of groups; in the Ascidia (Distaplia, Davidoff, '89), in amphibians (Salamandra, Rabl, '85; Rana and Triton, Schultze, '87; Rana and Necturus, Macallum, '91; Axolotl, Fick, '93; Diemyctylus, Jordan, '93; Salamander, Metzner, '94; numerous Batrachia, Carnoy et Lebrun, '97, '98, :00); in Aves (Pica, Mertens, '94), and in mammals (Mus, Hermann, '89, Holl, '93, Sobotta, '95). I have myself seen similar appearances in the germ cells of reptiles (Eutainia and skink), but have never studied them in detail. A like condition exists in the germ cells of numerous arthropods, according to Carnoy ('85). Karyospheres are certainly present in all the principal genera of Chilopoda (Carnoy, '85; Blackman, :01).

Chromatin-bearing nucleoli are not confined to animal cells, but are found in the cells of many plants as well. Their presence is vouched for in Spirogyra by Meunier ('86) and Moll ('93). Meunier says: "Nous ne craignons pas d'affirmer que le nucléole des Spirogyra réproduit fidèlement, dans ses traits essentiels la structure des noyaux les plus parfaits . . . quoi qu'il en soit, nucléole par position, noyau par nature, on ne peut lui refuser le nom de nucléole noyau, dans le sens attaché à ce mot par J. B. Carnoy." In diatoms (Lauterborn, '96) the nucleoli contribute to the formation of the spireme, and in Fritillaria (Heuser, '84; Strassburger, '84) a similar condition exists.

These pseudo-nucleoli vary considerably in their general characteristics, from small globules of pure chromatin, found in some cells, to the highly complex karyospheres present in Amphibia and Myriapoda. Of these the most complex type is probably represented in the spermatocytes of the Chilopoda, where this structure contains all of the essential elements of a nucleus, — chromatin, karyoplasm, and karyolymph. The same type also occurs in Protozoa (Actinosphaerium) and in plants (Spirogyra). In Amphibia there are a number of "nucleoli," but they still are characterized by the reticular structure. In the majority of these nuclei the chromatin elements have been shown to be derived from the "nucleolus," either entirely or in part.

It seems strange that the occurrence of phenomena observed by so many cytologists should not be credited or should be received only with scepticism by the majority of their co-workers, but such appears to be

the case. However, the conditions existing in Protozoa have not been generally known until quite recently, and in explaining the structures in question a great deal must depend upon our knowledge of their phylogeny. From the results of studies on Protozoa we learn that the occurrence of karyosomes, or of karyospheres, from which chromosomes are derived, is not an aberrant phenomenon in animal cells. On the contrary, these structures represent the primary condition in which chromatin occurs. For this reason we logically should not ask an explanation of their occurrence, but should rather inquire why the chromatin is ever arranged in a different manner. Our problem is to explain why the chromatin is arranged in a spireme rather than in a solid or granular mass. In the Protozoa a condition approaching the spireme exists for only a short time in the prophase (Hertwig, '99, Actinosphaerium; Calkins, '98[a], Noctiluca), while in the resting condition of the nucleus the chromatin is aggregated into one or more homogeneous or granular masses. In regard to the phylogeny of the chromosomes, as well as of other structures of the cell, sufficient knowledge is not yet possessed to warrant drawing any general conclusions. No comprehensive phylogenetic study of animal cells has ever been undertaken, and until such a study is accomplished, or until the series of facts accumulated by the work upon isolated types has become more complete, conclusions with any degree of certainty as to their accuracy cannot be drawn. Calkins's work upon Protozoa and his comprehensive discussion of the work of other investigators upon these unicellular animals has probably done more toward this end than that of any other author with which I am acquainted, but our knowledge of the relationships existing between the various parts of the cell in Protozoa and Metazoa is still very insufficient.

In Metazoa the aggregation of the chromatin into chromatin nucleoli or karyospheres seems to occur only in cells which continue for a long period without dividing. Thus they are much more common in egg cells during the growth period than in any other type of cell. They are of universal occurrence in the growing spermatocytes of Chilopoda, which in general characteristics, and in the duration of this growth period, resemble very closely the egg cells. Thus, I believe, we may conclude in a general way that the karyosphere is found only in cells which for a long period remain quiescent, i. e. do not undergo division. This conclusion holds good for all known cases where similar structures occur, somatic cells as well as germ cells.

### B. *The Accessory Chromosome.*

The literature upon the accessory chromosome has been reviewed with such detail in a recent publication by McClung (:02[a]) that it will not be necessary or desirable for me to consider it at any length. This element has now been found in three classes of the branch Arthopoda, viz., Insecta, Arachida, and Myriapoda. A structure in many respects similar to it, and apparently representing it, has also been described by Montgomery (:00) in the Protracheata (Peripatus). This chromosome displays a constancy of behavior in the spermatocyte divisions of insects which argues strongly for its great importance. This constancy is especially marked in the various families of Orthoptera. The element in the cells of this group is derived directly from a single spermatogonial chromosome — Sutton (:00, :02) in Acrididae; McClung (:00) in Acrididae and (:02[b]) Locustidae; de Sinéty (:01), in various Orthoptera — and according to the last two authors mentioned participates in but one of the maturation divisions. In these cells the accessory chromosome seems always to maintain its own individuality, and for this purpose it is at various stages in its early history contained in a vesicle separate from the other chromosomic vesicles which go to form the nucleus (Sutton, :00), while in the prophase of the spermatocyte it is at all times plainly recognizable on account of its less granular nature. For a considerable time it was supposed that it differed from the other chromosomes of the same period by the entire absence of the spireme condition. This conclusion is now found to be untenable, since during the spermatocyte prophase in Orchesticus and other locustids, the accessory chromosome assumes a spireme stage (McClung, :02[b]). The spireme of this element, however, differs from that of the other chromosomes inasmuch as the chromatin is less diffuse, and in its early stages so closely coiled upon itself that its true nature can be learned only by using high magnification. Later this thread thickens and unfolds, at this time resembling the ordinary segments in mid prophase, except in the fact that it is more homogeneous. Thus it is seen that this element does not differ so markedly from the other chromosomes as was at first supposed.

Regarding the function of the modified chromosome, two theories have now been advanced. Paulmier in his paper on Anasa puts forth the theory that the "small chromosome" represents characteristics which are being eliminated from the race. He bases these conclusions entirely upon the failure of the element to divide in one of the spermatocyte

divisions. Montgomery in his later papers adopts the conclusions of Paulmier, believing with him that it is a chromosome undergoing the process of elimination. McClung (:02[a]), in a paper dealing in detail with all of the reported observations upon the accessory chromosome, formulates an hypothesis which ascribes a very different function to this element. He maintains that the mere fact that the chromosome enters into the formation of only half of the spermatozoa would not necessarily indicate that the element is degenerating, and, in addition, that there are other facts which militate strongly against such a conclusion. The extreme nicety with which this element is excluded from contact with the others at all stages, and especially in the spermatogonia, would seem to indicate a very different and a much more important significance. This exclusiveness taken in connection with the fact that exactly one half the spermatozoa contain this element, suggests the theory that it has to do with the determination of sex, as this is the only respect in which the progeny are divided into two classes of equal number. Although no positive proof is advanced to support this theory, the author establishes, in a very logical manner, the probability of the accessory chromosome exercising such a function. It seems to possess all of the characteristics required of such an element. Definite proof of the function of this structure can be obtained, however, only by a study of the processes occurring in the fertilization of the egg.

My observations upon the accessory chromosome in Scolopendra have added very little to our knowledge of this element, except in so far as they demonstrate its wide distribution and the great similarity of its behavior in widely separated groups. Indeed, in all important particulars, the phenomena accompanying the development of this structure are identical in Chilopoda and Orthroptera, although in minor details the processes vary considerably. In both groups the element is derived directly from a single spermatogonial chromosome, and for this reason takes no part in the phenomena of synapsis. During the prophase, when the other chromosomes divide into four chromatids and form tetrads, this element, as would be expected from its origin, cleaves only once and then longitudinally. In the two succeeding divisions it is divided only once, and thus is present in but one half of the spermatids. The differences, although at times puzzling, are in reality slight and unimportant. Thus, at the time when all of the chromatin is aggregated in the karyosphere, the accessory chromosome cannot be distinguished, except in cases of the most favorable sections; but from the study of these thin, well-differentiated sections one is justified in saying that

even in the vesicle stage this element retains all of its ordinary characteristics. In the metaphase it can be distinguished from the other chromosomes both by its form and the different relation it bears to the poles of the spindle, it being connected by the mantle fibres to only one of these, while each of the other elements is connected with both poles. In the spermatids, instead of persisting for a considerable time, as in the Acrididae, it breaks down nearly as rapidly as the other chromosomes, and in a short time becomes indistinguishable from them.

These variations, as I have said, are but unimportant modifications of behavior, and do not represent such fundamental differences as seem to exist between the "small chromosome" (Paulmier) or "chromatin nucleolus" (Montgomery) in Hemiptera and the accessory chromosome in Orthroptera. If the observations of Paulmier and Montgomery concerning the origin of the bodies described by them are correct, it is indeed doubtful whether they represent the same structure as the accessory chromosome. The "chromosome $x$" of Protenor (Montgomery) would seem more closely to approach this modified chromosome in origin and later behavior.

Since the above was handed in for publication a paper by Miss Wallace (:05) has appeared, which contains still another hypothesis regarding the significance of the accessory chromosome. As a result of the maturation divisions of various spiders, the accessory chromosomes (two) pass to but one of the four spermatids derived from one spermatocyte, and only this favored spermatid completes the metamorphosis into a spermatozoön, whereas the other three degenerate. From preparations of the testes of Lycosa, collected in November, which I possess, I can say with certainty that no such degeneration of the spermatids occurs in this genus at least. In these preparations all stages of metamorphosis are represented, and there can be no doubt that all develop into spermatozoa.

### C. *Synapsis or Pseudo-Reduction.*

The term synapsis, introduced into cytological literature by Moore ('95), was employed by him to cover the period after the completion of the last spermatogonic division during which the chromosomes are reduced to one half the somatic number. "The transition from the first into the second spermatogenetic period is completed in the cells during the rest which follows the last division of the first. . . . The commencement of this metamorphosis is marked by an increasing fineness of the reticulum in the nuclei. After a while the nuclear network again grows

coarser and thicker, displaying at the same time a peculiar tendency to contract to one side of the nucleus, leaving a great clear space across which stretch numerous linin filaments." This grouping of the filaments at one side of the nucleus is in Moore's material characteristic of the period during which pseudo-reduction occurs, but it should not be considered a universal criterion of the synapsis stage. If I understand Moore correctly, the term synapsis is not meant to describe the massing of the chromatin spireme at one side of the nucleus, but to signify the fusing together of the spermatogonial chromosomes into pairs to form the spermatocyte chromosomes.

My observations upon Scolopendra, as shown in a previous paper (Blackman, :03), agree with the later ones of Montgomery (:00, :01) on Peripatus and Hemiptera, with those of Nichols (:02) on Oniscus, and with the much more convincing ones of Sutton (:02) on Brachystola, in demonstrating that the synapsis, or union of the spermatogonial chromosomes in pairs, occurs during the telophase of the last spermatogonial division. From observations upon stages immediately succeeding synapsis it is plain that this reduction of the number of chromosomes to form the number characteristic of the spermatocytes is accomplished by an end to end union of entire spermatogonial elements.

It appears to be the general conception that pseudo-reduction does not occur till a considerably later period. The process is usually said to take place in the early part of the active prophase of the first spermatocyte, when the chromatin is in the form of a fine spireme. This spireme is said by many writers to be a continuous thread involving all the chromatin of the nucleus, and one of the first changes noticeable in the early prophase is said to be the cleavage of this single thread into a number of segments equal to one half the number of spermatogonial chromosomes going to form it. Such, with slight modifications, is the conception entertained until recently by nearly all cytologists.

The reduction in the number of chromosomes in Scolopendra is not accomplished in this manner, but occurs, as I have said, during the spermatogonial telophase at the time the elements are becoming granular and elongated. There is at no time a continuous spireme in the cells of this animal. Upon the reconstruction of the nuclear membrane at the close of the long-continued telophase, the chromatin instead of existing in the form of a spireme, consists of a number of distinct segments equal to the number of chromosomes occurring in the succeeding metaphase. Reduction has already occurred, as will be seen by examining the accompanying figures. The chromatin threads are not closely massed

together during the early prophase, as is usual in insects, but are small in proportion to the size of the nucleus and are evenly distributed throughout the enclosing vesicle. This makes it possible to obtain an accurate count without any chance of error. That this process of pseudo-reduction is universal, at least in the arthropods, would seem probable from its occurrence in four of the classes of this branch (Crustacea, Myriopoda, Protracheata, and Insecta), and it may well be common to all sperm cells.

Perhaps the most interesting and valuable recent contributions to the question of synapsis are those of Sutton (:02, :03). This is true, both of the convincing manner in which he demonstrates the character of the union, and of the significance ascribed by him to the process. In the spermatogonia of Brachystola there are twenty-two chromosomes, exclusive of the accessory chromosome. The ordinary chromosomes show such constant relations in the matter of size that they may be readily separated into eleven groups, the two forming each group being of approximately equal size. Sutton shows that the two chromosomes of any given size conjugate during synapsis by an end to end union and during the division of the second spermatocyte are separated at this point of union.

The significance ascribed to this process is of the greatest interest. Following the earlier suggestion of Montgomery (:01), he believes that the two chromosomes uniting in synapsis represent similar characteristics, one being derived from each parent. Then by the reduction division it is brought about that no character possessed by the mother cell will be either duplicated or lacking in any one spermatid or mature egg. This serves as an explanation of the well-known fact that the nucleus of either spermatozoön or egg possesses all the necessary characters for the formation of a normal embryo (merogamy, artificial parthenogenesis). However, the chromosomes going to one cell are not all necessarily either maternal or paternal, and thus there arise chances of variation in the offspring proportional to the number of chromosomes characteristic of the cells of the organism. Thus the phenomena of the chromosomes is brought into correlation with the known facts of heredity and variation.

### D. *Formation of Tetrads.*

The literature upon tetrad formation and chromatin reduction has been so often and so thoroughly discussed that it will not be necessary for me to treat of it at great length. The observations seem in general to warrant the conclusion that in vertebrates the maturation divisions involve

two apparently longitudinal cleavages of the chromosomes. This view is supported by the observations of the following: Moore ('95) on elasmobranchs; Flemming ('87), Meves ('96), Carnoy et Lebrun ('98), McGregor ('99), Eisen (:00), Kingsbury (:02), Janssens (:01) on Amphibia; Lenhossék ('97), and Hermann ('89) on mammals, while it is opposed by those of very few authors indeed.

Montgomery (:03), following out the suggestion of Fick ('93), has demonstrated that in Plethodon and Desmognathus one of these divisions, while apparently longitudinal, accomplishes the same result as does the transverse division in invertebrates, i. e. the separation of entire spermatogonial chromosomes at the point where they united in the preceding synapsis. Thus the union of the chromosomes of vertebrates during synapsis is, in effect at least, a side to side union as distinguished from the end to end union characteristic of invertebrates.

In invertebrates — if we exclude Ascaris, which, on account of the nature of the chromatin bodies, naturally undergoes different changes — reduction is accomplished by one longitudinal and one cross division of the chromatin filament of the prophase. In this conclusion the following investigators agree: in Arthropoda Henking ('91), vom Rath ('92, '95), Toyama ('94), Rückert ('95), Paulmier, ('98, '99), Montgomery[1] ('98[c], :00, :01), McClung (:00, :02[b]), Blackman (:01, :03), P. Bouin (:03), and P. et M. Bouin (:02); in Mollusca Lee ('97), Griffin ('99), Linville (:00), Lillie (:01); in lower invertebrates Klinckowström ('97), Francotte ('97), van der Stricht ('96), and Griffin ('99).

The only investigators with whose work I am acquainted who oppose this view are: Wilcox ('95), Brauer ('92), and de Sinéty (:01), and the work of these three investigators has been discredited by other observations upon the same or closely allied materials. Wilcox asserts that the two spermatocyte mitoses accomplish a double transverse division of the chromosomes. Such is not the case in the western individuals of the same species, in which a longitudinal, followed by a transverse, division invariably occurs. De Sinéty, working upon the cells of several genera of Orthoptera, asserts that the two divisions are longitudinal. This also appears to be a mistaken conception, as pointed out by McClung (:02[b]). Appearances, which upon superficial examination might lead to this view, are occasionally met with in orthopteran mate-

[1] In an earlier paper Montgomery reported the occurrence of two cross divisions as universal, but in his subsequent articles he has entirely withdrawn this statement, and now believes that reduction is always accomplished by one longitudinal and one cross division.

rial, but when studied closely a different interpretation must always follow. In the spermatocytes of Scolopendra I believe it is impossible to arrive at this conclusion, however strong a preconception the observer may have had. The tetrad figures accompanying this article can by no possibility be logically interpreted as representing anything but a longitudinal, followed by a transverse division of the chromosome. In the interpretation of the chromosomes of the first spermatocyte and in the sequence of the succeeding divisions, I am gratified to note that P. Bouin, working upon other species of Myriapoda, agrees with my conclusions for Scolopendra.

The forms of tetrad which are of most common occurrence in the arthropods are modifications of the cross, double-V, and ring figures found in Anasa (Paulmier, '98) and in Hippiscus (McClung, :00). It is probable that the tetrads found in all of the other insects are obtained by a greater or less modification of the same process. Such is evidently the case in copepods (Rückert, '94; Häcker, '92), and in Gryllotalpa (vom Rath, '92), and it seems also to be true of other invertebrates, — Thalassema and Zirphea (Griffin, '99), Unio, (Lillie, '98), etc.

The typical tetrad of arthropods, as exhibited in the Insecta (Hemiptera, Paulmier; Orthropera, McClung) and in the Myriapoda (Scolopendra, Blackman), is produced in the following manner: The chromatin segments of the reduced number, as they arise from the spireme stage (Insecta), or from the aggregated segments of the karyosphere (Myriapoda), are long slender threads of a granular character. Each thread very quickly splits longitudinally, giving rise to two long slender segments extending parallel to each other. Very shortly after this longitudinal split becomes visible, indications of the second division at right angles to the first, may be seen. The further changes of the chromatin segments in insects are not essentially different from those of Scolopendra, which have already (p. 29) been described.

While great unanimity exists among workers upon the germ cells of invertebrates with regard to the nature of the tetrads, there is still considerable dispute concerning the sequence of the divisions. By far the greater number, however, agree that the longitudinal division comes first and is succeeded by the cross division. Rückert, Häcker, McClung, Blackman, and P. Bouin have arrived at this conclusion for arthropods, and Lee, Linville, Griffin, Klinckowström, and Francotte agree with them for other invertebrates. The opposite view — i. e. that the reduction, or cross, division precedes — is held by the following: vom Rath, Henking, Paulmier, and Montgomery for arthropods, and Lillie for

molluscs. In arriving at this latter view the criterion invariably used is the appearance and behavior of the elements during the two mitoses. But during the metaphase the chromosomes are often so compact that the planes of the cleavage shown in the prophase are entirely obliterated, and therefore the manner of division cannot be determined with certainty. An example of the likelihood of misinterpretation of the nature of these divisions is shown by Griffin ('99) in Thalassema. Here the first division is evidently longitudinal, and upon superficial observation the second also appears to be of the same nature. But when the phenomena observed in the prophase are considered, it is evident that this cannot be true, since an indubitable transverse cleavage was to be seen at that stage, and upon further examination the first impression is shown to be false, for the second division is in reality a reduction division. Thus I believe it is to the prophase of the first spermatocyte that we should look for the evidence upon which to establish our conclusions regarding the sequence of the divisions. In this I agree with McClung (:00), who says, "Too much importance cannot be laid upon the necessity for a thorough understanding of the early formative periods in the history of the first spermatocyte chromosomes." In all of the investigations with which I am acquainted it has been shown that the longitudinal cleavage is the one first made manifest in the prophase. I believe it is but logical to conclude that the longitudinal division is completed by the first spermatocyte mitosis, especially since this has been shown to be the case in a great number of cells. Of course it is possible that this process varies in different animals, but it is not probable, for if the sequence of the actual divisions varies, we should naturally expect the prophase phenomena to vary in like manner. As I have already said, the longitudinal splitting of the chromatin segments invariably occurs first. When these facts are all considered, I believe one is justified in believing that in all Invertebrata the first maturation division is an equation division, the second a reduction division.

### 2. Cytoplasmic Structures.

#### A. *Centrosome and Centrosphere.*

During the last decade no structure of the cell has been the subject of more controversy among cytologists than the centrosome. This is not extraordinary when we consider its extremely small size, its great variation in form, its apparent difference in different classes of cells and even in different individuals of the same general class, and the striking irreg-

ularity which it often exhibits in its reaction to various stains. When we compare the simple granule representing this structure in the male cells of insects, with the comparatively immense, complicated structure in the eggs of Limulus described under the same name by Munson ('98), it must be seen at once that either the centrosome exhibits a great dissimilarity in different cells, or very different structures have been included under one term. As a matter of fact there is much discussion as to what structure shall be designated by the term centrosome. Wilson ('95) expresses this as follows: "The word 'centrosome' is at present used in three different senses, being applied (1) by Boveri to the entire central mass of the aster exclusive of the rays ("astrosphere" of Fol, "centrosphere" of Strassburger), (2) by Strassburger and others to a smaller dark body often found within the centrosphere, and (3) by Heidenhain to the individual granules of which this dark body is made up." Since this was written, many new facts in connection with the centrosome have been discovered, but the confusion in the terminology has not been rendered less marked by these observations. Indeed, owing to the greater number of individuals engaged in these studies, the confusion of terms seems to have increased rather than lessened. This fact is greatly to be deplored, for in making a comparison of apparently identical structures described under various names by different authors one is often at a loss in attempting to correlate the results.

Boveri (:01), in his work upon the nature of the centrosome, gives at various places the characteristics by which one may distinguish the centrosome and the centriole. The centrosome is in his view that part of the structure found at the centre of the aster during mitosis which is present at all stages of cell division and divides to form the centres of the daughter cells. He gives as a rule: "Ein Körper, an den die Sphärenradien direkt herantreten, ist das Centrosoma." Division of the centrosome is always preceded by the division of the centriole. "Das Centriol teilt sich beträchtlich früher als das Centrosom." This division generally occurs at the time of the formation of the equatorial plate, but may take place earlier (Echinus): "Die Teilung des Centrosoms selbst scheint dagegen normaler Weise nirgends früher als in der Metakinese zu beginnen . . . Doppelkörner zur Zeit der Aequatorialplatte oder früher, werden also mit grosser Sicherheit als Centriolen in Anspruch genommen werden dürfen." The centrosomes are, "Die grössere der beiden in einander geschalteten körperlichen Differenzierungen im Centrum der Sphären." The centrioles are the smaller central bodies and are "von so extremer Kleinheit, dass sie selbst in den grössten Zellen, wie den

Eiern, auch mit den stärksten Vergrösserungen nur als kleine, nicht weiter analysierbare Pünktchen erscheinen."

What structure in Scolopendra heros answers the requirements of such a centrosome? It is at once very plain that the only structure of this class which is present at all times is the deeply staining body. This is to be found at all stages of the development of the testicular cells, and does not vary at any time in its staining reactions. In the prophase, when the centrosomes are moving in opposite directions upon the nuclear membrane, there is no sign of any outer sphere about them. The astral rays converge toward these small dark bodies and can be plainly seen to come in direct contact with them. Later in the prophase, when a surrounding zone is beginning to appear, the rays can be plainly seen to penetrate this zone in their course toward the central body. In the prophase of the second spermatocyte this structure lies apparently naked in the cytoplasm, and a like condition also exists in the telophase of the second spermatocyte and in the early spermatids. But "das wichtigste Kennzeichen" is the relation of the centrosome and the astral rays. As quoted above, the body with which the rays come into direct contact is the centrosome. It has been shown that in the prophase the rays are directly continuous with the dark central bodies, even after the surrounding sphere has become visible. The same is true in the late metaphase and anaphase of the small spermatocyte. Here, when the outer sphere has decreased in size, the rays can plainly be seen to converge in the central body. During the early metaphase, however, and in the telophase they cannot be traced through the outer zone on account of its much larger size and greater density at these periods.

The centrosome according to Boveri should not begin to divide before the metaphase, and should not complete this separation until considerably later. The central bodies in the spermatocytes of Scolopendra show their first sign of division at the time of their divergence in the prophase, but this division is never completed before the anaphase and in the small spermatocytes does not occur until even later. According to Boveri, therefore, these bodies should be called centrioles. But centrioles are so extremely minute that even in the largest cells and under the highest magnification they appear as mere points, incapable of further analysis. The central bodies in my material do not fulfil this requirement as well as they do that of Boveri's centrosome. They are not small points incapable of further analysis, but are of considerable size, and at all times, except during the vesicle stage, when completely inactive, of a granular or spongy consistency, as is the case with the central body in

Toxopneustes (Wilson, '95[b], :01[a], :01[b]) at various stages. According, then, to Boveri's own criteria — i. e. the persistence of the body and its relation to the astral systems — *I believe I am justified in identifying the small deeply staining body at the centre of the asters as a centrosome.* The only characteristic which does not fit into his definition of the centrosome is the time of division. When the great variation in the morphological characteristics exhibited by this organ in different cells is considered, and the great variation in the physiological processes as well, I believe that no conclusions of such fundamental importance as the identification of this element should be based upon its time of division.

I am convinced that the less dense sphere surrounding this centrosome, which I have called the centrosphere, represents a different substance. It is probable, according to my observations, that this substance is a reserve supply of archoplasm in a highly condensed form. This archoplasmic material is of a similar nature to the ordinary archoplasm of the cell during that period of the prophase in which it is apparently dissolved in the hyaloplasm, except that it is probably in a more concentrated form. This centrosphere substance is used when occasion demands in the formation of new fibres or in the elongation of those already formed. Appearances which strongly support this conclusion may be observed in the first mitosis of the small type of spermatocyte, where the centrosphere is large and conspicuous, while in the succeeding stages it is very much smaller. During the interim the centrosomes have moved apart and the spindle has been modified in such a manner as to give rise to the apical point. Considerable of the reserve archoplasm contained in the centrosphere has evidently been withdrawn from this structure and has been used in the production of the set of fibres connecting the centrosome and the apical points. Furthermore in the telophase, when the chromosomes have been drawn apart and are collected in masses at the opposite ends of the cell, the centrosphere again increases in size. This is evidently accomplished by the retraction of the mantle fibres and their conversion into latent archoplasm.

This is the only way in which I can interpret the observed facts, and if this explanation is correct, the centrosphere should not be considered a component part of the highly specialized centrosome, but should more properly be classed with the less differentiated archoplasm. These observations, while in themselves quite different from those of Lillie ('98), would seem to lead to the same conclusions that he reached in studying the formation of the second polar spindle in the egg of Unio. He observed that the second maturation spindle arises from the material

of the inner sphere (centrosome of Boveri), which is evidently analogous to the centrosphere of Scolopendra. He also interprets the central body as the centrosome, because it serves as the centre of the astral radiations and because it divides to form the centres of the next generation of cells. Lillie's observations are very similar to those made independently by MacFarland upon another mollusc (Diaulula), but his interpretation is quite different. Although MacFarland shows that the spindle arises from the substance of the inner zone, still he calls this structure the centrosome. Thus the entire spindle is formed directly from the centrosome! It is of course conceivable that a part of the centrosome itself should go to form the astral rays, however improbable it may seem; but to conclude that the whole spindle arises thus seems absurd.

In Scolopendra the history of the centrosome and centrosphere absolutely precludes such an interpretation. It is, I believe, impossible that the centrosphere should arise directly from the centrosome, for the dark central body is at all times visible. Rather the centrosome seems to exert a formative power upon the archoplasm which causes the great mass of this substance to be converted into fibres at the beginning of mitosis. A part, however, is collected about the centrosome as a reserve supply (centrosphere), and this supply is used in the elongation of the already formed fibres or in the formation of new ones, as occasion demands.

In the egg of Ascaris Boveri ('88) reported that the centrosome during metakinesis consists of a rather large sphere containing a central granule. This entire structure is obtained by the elaboration of the centrosome of the prophase, which at that time is but a simple granule. The centriole found in the metaphase would seem to be a direct differentiation product of the surrounding sphere or centrosome. Quite similar appearances were found by the same author in a later study of the egg of Echinus. However, Brauer ('92) found that in the spermatocytes of Ascaris the central granule is present at all stages, and is thus not a derivative of the sphere, or centrosome of Boveri, which surrounds it during the metaphase. In addition Kostanecki und Siedlecki ('96) announce that in lightly stained preparations of Ascaris the astral rays may be traced to the central granule, and thus, even by Boveri's most important criterion, this body is the centrosome. Practically the same conclusions have been reached by other investigators upon material obtained from various sources. In Chaetopterus, Mead ('98) identifies "the minute dark granules in the centres of the asters as centrosomes because they persist and by successive divisions furnish the centrosomes of each

cleavage spindle up to the eight-cell stage." On equally good grounds, Griffin ('99) decides that, in Thalassema and Zirphea, "the black focal granule is functionally here the true centrosome, as understood by Boveri — the single permanent cell organ, which forms the dynamic centre of the cell and multiplies by division to form the daughter cells." A like position is taken by Wheeler ('95, Myzostoma), Lillie ('98, Unio), Linville (:00, Limnaea), Coe ('99, Cerebratulus), and Kostanecki (:02, Cerebratulus).

In the sperm cells of insects it is not so difficult to locate the centrosome, for there is at the centre of the aster (which is usually not well developed) a simple granule toward which the rays converge. This granule is, as a general thing, contained in no centrosphere or similar structure, and hence is indubitably the centrosome. Such structures are figured by the following authors: Platner ('86, Lepidoptera), Henking ('91, Pyrrhocoris), Toyama ('94, Bombyx), vom Rath ('92, Gryllotalpa), Montgomery ('98 to :01, Hemiptera), Paulmier ('99, Anasa), McClung (:00 to :02[a], Acrididae, Locustidae), Sutton (:00, Brachystola), de Sinéty (:01 Orthoptera).

On the other hand, Boveri's view that the inner zone is the centrosome is supported by Fürst ('98, Ascaris) and MacFarland ('97, Diaulula and other gasteropods). The observations of Smallwood (:01) upon Bulla would seem also to agree in some points. Wilson ('95[b], Toxopneustes), and Conklin (:02, Crepidula) find a large reticular sphere at the centre of the aster at certain stages, in which the homologue of the centrosome is very hard to distinguish. In the early stages, however, Wilson finds "one or two extremely minute granules (centrioles), which stain deep blue in the haematoxylin," contained in an irregular mass which is colored by the Congo red. He believes this mass with its centriole corresponds to Boveri's centrosome. In this paper Wilson concludes that the central granule ("centriole") "is formed endogenously in the central mass." Such is certainly not the case in Scolopendra, where this central mass or centrosome may be seen at all periods of the cell's development and indeed is the only part of the structure at the centre of the radiations which shows this persistence. From the phenomena observed in Scolopendra it would seem, on the contrary, that the centrosphere and aster are rather exogenous formations brought about by the influence which the centrosome exerts upon the archoplasm of the cell.

In a later paper Wilson (:01[a]) states his belief that there are "good bases for the conclusions that centriole, centrosome, and aster are but concentric differentiations of a structure that is essentially a unit."

That the structures often act as a unit during the stages of active division cannot be disputed, but neither can it be denied that at other times centrosome and archoplasm are entirely distinct structures. During the division of the chromosomes and during cytoplasmic cleavage in Scolopendra the centrosome and aster do indeed appear to act as a unit, but that their origin is quite different is shown by a study of the prophase stages, when it is seen that the centrosphere does not arise by the enlargement of the centrosome, a new centrosome being formed endogenously, but that the sphere and aster arise by the transformation of the already existing archoplasmic masses. Thus, while aster, centrosphere and centrosome are a unit in one sense (in their behavior during active mitosis), the aster is not formed by the growth or direct transformation of the centrosome as described by MacFarland, although it may have its origin from the substance of the centrosphere (Lillie). But in this case the centrosphere is not of direct centrosomic origin, but is obtained from the previously existing archoplasm or by the differentiation of the cytoplasm.

From these considerations relative to the morphology of the centrosome, I believe the conclusion may be drawn that there are possibly three, but probably only two, general classes of structures which form the centre of the astral systems: that described by Wilson (Toxopneustes) and Conklin (Crepidula), and those reported by Boveri (Ascaris), Lillie (Unio), Mead (Chaetopterus), etc. I am convinced that no really essential difference exists between the centres of the asters as described by Boveri and MacFarland on the one hand and those reported by Mead, Lillie, and Linville, and observed by myself in Scolopendra on the other. It is evident that the discrepancies which appear to exist are due largely to differences of interpretation.

*The Centrosome as a Cell Organ.* — The question as to whether the centrosome shall be considered a cell organ or not seemed at one time very likely to be answered in the affirmative; but it is now in a very unsettled condition. While many observations have been accumulated which prove beyond dispute that in some cells at least, and possibly in a majority, the centrosome is one of the permanent morphological elements of the cell, and therefore should be considered an organ of perhaps equal rank with the nucleus, in other cells this is apparently not the case. In these at certain stages no structure can be found which answers to the requirements of a centrosome. This has led many to the belief that the centrosome is not a true organ of the cell, but merely a transitory appearance caused by the activities of other parts of the cell. In con-

sidering these diametrically opposite conclusions it must be remembered, as Mead ('98) has pointed out, that one is based upon undisputed observed facts, while the other is based upon negative evidence. "To maintain that the centrosomes are absent simply because they are not demonstrable is, of course, to base an assumption upon negative evidence, —a procedure especially dangerous when applied to the centrosome, inasmuch as this structure is, at best, very minute and comparatively difficult to demonstrate, even when its exact position is indicated by the presence of an aster."

By far the hardest blow to the theory of the continuity of the centrosome has been given by the new school of experimental cytologists. The experiments of Hertwig ('96), Morgan ('96), and Wilson (:01[a] and :01[b]) upon Echinoderm eggs treated with solutions of various salts and poisons seem to have proved beyond doubt that functional centrosomes may arise *de novo* in unfertilized eggs. It is not my purpose here to discuss these experiments; but one cannot read the recent experimental work of Wilson (:01[a]) without being convinced that centrosomes really may arise either in close relation to the nucleus or free in the cytoplasm. Under the same general head may be classed the observations of Mead upon the egg of Chaetopterus. He finds that when the egg is removed from the body fluid and placed in sea-water a number of asters soon appear. These asters gradually fade away, with the exception of two, which persist and increase in size. Small bodies appear in their centres which serve as the maturation centrosomes. At first glance these observations would seem to disprove the universal persistence of the centrosomes, but Wilson (:01[b]) assures us that this is not necessarily true. While the centrosome may not persist as such, it is possible that centrosome substance capable of producing a centrosome is present in the cell at all times, from which, as occasion demands, centrosomes may arise apparently *de novo*.

These experimental studies have left the question of the origin of the centrosome in such an unsettled state that I believe a further discussion at this time is useless. No universal conclusions can as yet be drawn concerning the rank to be accorded to this structure among the organs of the cell. In the testicular cells of Scolopendra heros the conclusion would seem to be justifiable that the centrosome is as truly a cell organ as is the nucleus. A similar conclusion has been reached in many other cells as well; but the evidence to the contrary brought forward by Lillie and others, and the even more conclusive evidence furnished by the experimental work of Hertwig, Morgan, and Wilson, should also be

admitted. When these results are considered, it must be acknowledged that at present our knowledge of this cell structure is not sufficiently full to afford a basis for any general conclusions. I believe it is to the experimental work of the future that we must look for the solution of this and many similar problems in cytology, although of course results obtained by such abnormal methods must be interpreted with extreme caution when considering general cell processes.

One point in Wilson's work which may prove to be of great interest is the significant fact that in the eggs treated with magnesium chloride both archoplasm and chromatin show a decided similarity in their behavior to analogous structures in the cells of Protozoa. He calls attention to the likeness existing between the structure of the aster in his material and the corresponding structure described by R. Hertwig in Actinosphaerium, and also shows that in one series of eggs the chromatin is aggregated in a mass, and in other ways behaves in a manner very much as in Protozoa. From his work it would seem that under abnormal circumstances the cell shows a decided tendency to revert to the primitive type.

The first investigators to assign any important function to the centrosome were Boveri ('87[a], '88, p. 754, '88[a]) and van Beneden et Neyt ('87, p. 279), whose accounts, while differing in several respects, agree in the essential conclusion that this structure is the "dynamic centre" of the cell. In this conclusion the observations of many later cytologists concur. Concerning Thalassema and Zirphaea, Griffin ('99) says: "By a careful study of these processes, the impression is most strongly conveyed that throughout all these stages the centrosome is the cause rather than the mere expression or by-product of the aster formation. This is especially clear in late anaphase, where the centrosome deserts the old system, and moving to a different locality furnishes there the stimulus to the formation of a new one." Phenomena very similar to these have also been observed by MacFarland ('97, Gasteropoda), Coe ('99, Cerebratulus), and Lillie ('98, :01, Unio). My results agree with these in showing indubitably that the centrosomes are not a chance product derived from the forming aster, but may be more justly said to be the causal, or at least the directive, force in the formation of the astral system.

It has been seen that the first change in the prophase of the spermatocyte concerns the centrosome. This is more marked in the large type of the spermatocyte than in the smaller, but nevertheless may be observed in both. This fact may well be taken as supporting the hypothesis of Boveri and van Beneden that the impulse for cell division is given by the centrosome.

One of the most important properties of the centrosome is said to be its influence over the separation of the chromosomes and over cytoplasmic cleavage. In considering the separation of the chromosomes in Scolopendra, it must be admitted that very little power is necessary for its accomplishment; for, as we have seen, during certain prophase stages the chromatids are already practically distinct bodies with well-marked spaces existing between them. They are at this stage apparently very loosely bound together by linin fibres. Later, when the chromatin becomes of a liquid or viscid consistency, it is generally admitted that these cleavage planes still exist, although they are masked by the greater condensation of the elements. It is therefore probable that the coherence of the various chromatids of the tetrad may be very easily overcome. Indeed it is conceivable that in some cases it may require no external influence to perfect the division. In Scolopendra the separation of the halves of the chromosomes occurs at the time of the lengthening of the cell, and is apparently inaugurated, not by the contraction of the mantle fibres of the spindle, but by the divergent movements of the centrosomes. After the division of the chromosomes and when they have very perceptibly moved apart, the mantle fibres contract and draw them farther toward the poles. Until the separation is well marked the distance between chromosomes and centrosomes is apparently not at all diminished, but later the contraction of the mantle fibres is shown by the approximation of these two classes of elements and by the enlargement of the centrosphere.

### B. *Archoplasm.*

*Persistence of Archoplasm.* — The behavior of the archoplasmic structures in Scolopendra supports in many particulars Boveri's well-known archoplasm hypothesis. During all stages of continued mitotic inactivity in Scolopendra the presence of definite archoplasmic substance is shown by special aggregations of a granular or reticular substance much resembling the ordinary cytoplasm but differing from it in several important particulars. This archoplasm is distinguishable from the unmodified cytoplasm by the closer aggregation of its component granules and by its different reaction to stains. These masses are most conspicuous during the growth period of the spermatocyte and during the vesicle stage, but they are also quite marked in the early stages of the spermatid.

At all stages immediately preceding and succeeding mitosis, masses of archoplasm are not to be found, but the presence of archoplasm is shown by the different character of the cytoplasm. It is plainly evident that it

is, at these times, in a very different condition from that in which it occurs during cell rest. As I have already stated, from appearances noted in the first spermatocyte (large type), the first change in the prophase consists in the disintegration of the archoplasmic zone surrounding the nucleus. The material becomes dispersed throughout the cell, causing cytoplasm in all parts of the cell to stain in a uniform manner. This change is due either to the solution of the archoplasm in the more fluid hyaloplasm, or to its breaking up into invisible particles, which become uniformly distributed. As a result of this process, the only visible evidence at this time of the presence of archoplasm is furnished by the slightly darker color of the hyaloplasm.

This state of solution or dispersion is but a stage in the transformation of the granular archoplasm of the resting stage into the fibrillar archoplasm of active mitosis. Convincing evidence of this fact is seen in the early stages of mitosis in the various cells. The growth of the astral rays of the first spermatocyte, large type, advances from the centrosome outward. As the archoplasm in solution is converted into astral rays the inter-reticular portions *pari passu* stain less deeply, and thus assume the appearance characteristic of hyaloplasm. This is especially noticeable during the rotation of the spindle. The astral rays when first formed in the early metaphase involve only the third of the cell nearest the spindle. The hyaloplasmic areas of this portion of the cell thus become transparent, while those in the ends of the cell where the astral rays have not as yet penetrated still show the deeper stain characteristic of the early prophase. By the rotation of the spindle the centrosomes come into closer relation with these end regions, so that in the early anaphase all parts of the cell are penetrated by astral rays. Meanwhile the inter-reticular areas in all regions have lost their contained archoplasm and are transparent. Stages in this transformation have been represented in Figures 32–35 (Plate **3**) and 144 (Plate **8**).

The prophase of the first spermatocytes of the small type likewise affords evidence that the archoplasm undergoes a kind of solution while being transformed into astral rays. In the small cells the archoplasm is not, as in the large spermatocytes, distributed throughout the entire cell at the beginning of the prophase, but collects at one side of the nucleus in a granular mass. This condition continues until the chromosomes have become quite dense. The next change occurs when the centrosome migrates to the nuclear membrane. At this time, and during the later separation of the centrosomes, the archoplasm gradually becomes dissolved and converted into astral rays. It is not all dissolved at once, as

in the large spermatocytes, but is gradually broken down, as the astral rays become more and more marked. The first change in this transformation is noticed in the region immediately surrounding the centrosome. Here the archoplasm becomes less granular, more transparent, and astral rays, faint and short, appear. As the number and length of these increases, more and more archoplasm is dissolved and becomes transformed, until, at the opening of the metaphase, none remains in the granular condition (Plate **5**, Figs. 57–59; Plate **9**, Figs. 154, 155).

The phenomena in the early stages of the second spermatocyte are similar to those occurring in the first spermatocyte of the large type. In another connection is mentioned evidence furnished by the spermatids, showing that in these also the archoplasm undergoes similar changes. Here the origin of the axial filament is seen to be similar to that of the astral rays at other stages. Both arise in connection with the centrosome, and both are of archoplasmic origin.

I think there should be no hesitation in concluding that in Scolopendra the archoplasm is a distinct substance, and that it is derived, in part at least, from the archoplasm of the parent cell. This is undoubtedly true of all generations of the cell after the spermatogonia, for the presence of this modified cytoplasm can be detected at all times. However, it certainly undergoes very marked changes in its morphological, and perhaps in its chemical, nature; first appearing in the granular form, then in the diffuse or dissolved condition, and finally in the fibrillar.

Thus, the conditions in Scolopendra would seem to realize Boveri's contention that the archoplasm is a modification of the cytoplasm and that the astral rays are different from the cytoplasmic reticulum. In all stages of mitosis the cytoplasmic reticulum in Scolopendra remains entirely distinct from the astral radiations, except in the region occupied by the spindle, where no reticulum occurs, although the structure of the reticulum is often obscured by the astral rays.

This view of the persistence of the archoplasm as opposed to the older hypothesis (Bütschli, '**76**) of fibrillar persistence is supported by the observations of many of the later investigators. Griffin ('**99**) and Coe ('**99**) find that the new asters as they arise around the divided centrosome are entirely independent of the old ones, which still converge toward the old position, even though the centrosome and new aster have moved away. Similar results have been obtained by MacFarland ('**97**), Lillie ('**98**), Smallwood (:**04**), and others. The fact that the astral rays persist in the absence of the centrosome also shows, I think, that they are definite structures and not due merely to diffusion currents. As the

new aster grows, the old one gradually breaks down, and its substance is probably used in the building up of the new system.

In his later papers Boveri ('95) admits that the archoplasm may not persist in the form of a definite body, but still may exist as a distinct archoplasmic substance distributed throughout the cell, or that it may arise as a differentiation of the cytoplasm having no longer any connection with the reticulum. In Scolopendra at all early stages of mitotic activity the archoplasm is in the second form, for while it still undoubtedly remains as a distinct substance, it is dispersed throughout the whole cell.

*Nature of the Astral Systems.* — It is the tendency of many of the later workers upon the nature of the astral rays to regard them as the expression of chemico-physical diffusion currents centring normally in the centrosomes. This view was first expressed by Bütschli ('76), who in a later paper ('92) expresses the opinion that the astral rays are merely the optical sections of the walls of the alveoli which are arranged around the centrosome in a radiating manner. Other workers upon eggs in which an alveolar structure of the protoplasm is undoubtedly present are inclined to adopt a similar or slightly altered view. Wilson (:01[a]), in his papers on chemically fertilized eggs of sea urchins, has come to the same conclusion, and Conklin (:02) has reached similar results in Crepidula. Petrunkewitsch (:01), working principally upon Strongylocentrotus, also concludes that the substance of the astral rays "ist die Substanz der Wabenwände; ein besonderes Archoplasma, oder Kinoplasma, oder wie es auch heissen mag, existirt nicht, wenn auch eine chemisch andere Beschaffenheit der 'Strahlen' nicht ausgeschlossen ist."

All of these authors have worked upon material in which the structure of the protoplasm is of the coarse alveolar type, often distorted and obscured by masses of yolk substance. It is evident that both of these characteristics would tend to obscure the nature of the astral rays if these were structural fibres. No convincing evidence has ever been given that a special archoplasmic substance is not present at the nodal points of the alveoli, while the presence of such material has been maintained by many, notably by Boveri ('95) and by Wilson in his earlier papers. In the more finely alveolar and in the so-called reticular cytoplasm, such as occurs in the male cells of Scolopendra, the astral rays are not so much obscured by other structures as in the coarser form of protoplasm; they appear as very fine, but in favorable cases definite, filaments, which are entirely distinct from the true cyto-

plasm. Such may likewise be the case, it appears to me, with regard to the same structures in cells which show a more marked alveolar nature. It seems probable that in these cells the astral fibres follow the course of the alveolar boundaries, and in the fixed material cannot be differentiated from the unmodified cytoplasm, i. e. alveolar walls, through which they pass. My observations relative to the question, whether the astral rays are of a fibrillar character or merely the visible indication of diffusion currents, all point to the former condition. That the astral rays in many eggs at least are not diffusion currents, but more permanent structures, would seem to be demonstrated by the observations of MacFarland ('97), Griffin ('99), Lillie ('98), Coe ('99), and Smallwood (:04), who show that the old astral rays still persist for a considerable time and continue to converge toward the same point, after the divided centrosome and new asters have moved away and taken up a new position. If the rays were mere diffusion currents, it seems highly improbable that they would persist so long. On the other hand, if they are fibres, it would naturally require considerable time for them to be broken down and their substance redistributed around new centres.

When we inquire into the function of the aster, we immediately find ourselves concerned with the problems of cleavage, so closely is the aster associated with this phenomenon. I do not, at this time, wish to enter upon the question of the mechanics of mitosis, since already many explanations, more or less unsatisfactory, have been attempted, and it is evident that our knowledge of mitosis is not yet sufficiently complete to serve as a basis of a theory which will explain all known phenomena of cytoplasmic division. However, as I have shown in the body of this paper (p. 50), my observations lend themselves more readily to the older, well-known hypothesis of cell division by fibrillar contractility. I have considered the subject in sufficient detail at that place, and will not enter into further discussion of it here.

### 3. Spermatid Metamorphosis.

The metamorphosis of the spermatid, while probably not as important from a theoretical point of view as the phenomena connected with the spermatocyte division, nevertheless offers many points of interest and has been the subject of much careful investigation. It is not my purpose to review the literature in any extensive manner, for within the last few years this has been done by a number of writers, the most notable

recent papers on this subject being the summary by Meves (:02[b]) and the chapter on spermatogenesis in Korscheldt und Heider (:02). Instead, I shall compare certain phenomena observed in Scolopendra heros with those observed in other related animals.

In a general way the development of the spermatid into the spermatozoön in Scolopendra shows phenomena similar to those observed by Collin (:01), Tönniges (:02), and M. P. et Bouin (:03) in other genera of myriapods. However, in many details it differs quite markedly from the conditions observed by these authors. After a hasty study of the process in several species of Lithobius, Geophilus, and Scutigera, I am of opinion that this is not entirely due to differences of interpretation. Nevertheless, it is my belief that there is greater uniformity than would appear from the accounts hitherto published. This conclusion would seem to be corroborated by the differences in results reached by Collin and M. P. et Bouin working upon the same material prepared in the same laboratory. In later studies upon the spermatogenesis of myriapods, I hope to return to this subject, and to attempt, at least, to correlate the processes in different genera of chilopods. In view of the considerable differences existing between the spermatocytes of two species of Scolopendra (S. heros and S. subspinipes), and especially those exhibited by the two classes of cells in the single species S. heros, it would not be surprising if the cells of animals belonging to different families of myriapods should show considerable variation in their development.

### A. "*Nebenkern.*"

One of the most interesting facts about the spermatids of myriapods is the complete absence of a true Nebenkern, which has been shown to be characteristic of certain stages in the transformations of the sperm cells of insects. The word Nebenkern when used in the original sense, Bütschli ('71[a]), refers to that body in the spermatid which is produced, at least partly, by the aggregation of the spindle remnants, and in later stages gives rise to the inner tail envelope of the spermatozoön. In Scolopendra, as we have seen, the spindle remnants are very often cast out of the cell in the late telophase of the second spermatocyte, and are never aggregated into a structure comparable to the Nebenkern of insects. The substance surrounding the centrosomes and the base of the axial filament is not homologous with the Nebenkern, for, while it arises from the archoplasm, its origin and behavior are quite different from that of the Nebenkern in insects. In insects, according to the researches of

Butschli ('71[a], '71[b]), La Valette St. George ('86, p. 9), Platner ('89), Henking ('91), Wilcox ('95, '96), Erlanger ('96[b]), Paulmier ('99), Baumgartner ('02) and others, the Nebenkern results from the direct metamorphosis of the interzonal filaments. The remnants of the spindle are not dissolved and subsequently reassembled to constitute this body. Moreover in insects, the centrosome is at the pole of the cell opposite to the Nebenkern at the time of the origin of that body.

In Scolopendra the archoplasmic mass is quite different in origin. It first appears upon the side of the cell opposite that at which the mid-body was last seen, and it always arises in very close relation with the centrosome. In fact, it partakes more of the nature of an idiosome than of a Nebenkern. Moreover, in Scolopendra it does not arise directly from the spindle remnants, — i. e. from archoplasm in the fibrous condition, — but from archoplasm which has been dissolved and now reappears in the granular condition characteristic of so-called resting cells, and at a stage later than that at which the interzonal filaments disappeared. Therefore I speak of this mass not as Nebenkern or idiosome, but as undifferentiated archoplasm. It cannot be the Nebenkern because of its origin, and I do not consider it a true idiosome because of its very irregular shape, variable size, and undifferentiated character.

### B. *Faserkorb.*

While none of the workers upon myriapod spermatogenesis have described a Nebenkern in the spermatids, Tönniges (Korschelt und Heider, :02, p. 527) finds in Lithobius, at the stage when the nucleus is beginning to elongate, a differentiation — apparently a condensation — of the protoplasm, which is probably comparable with the so-called Faserkorb (Schwanzmanschette) of mammals described by Meves and other authors. He also calls attention to its similarity to the Nebenkern of Paulmier ('99), notwithstanding the difference in origin. "Das Bild (Nebenkern) zeigt jedenfalls mit den bei Lithobius auftretenden eine sehr grosse Aehnlichkeit." The structure described by Tönniges is the same as that shown in my Figures 96–109, which in Scolopendra is derived from that portion of the archoplasm which remains after the greater part of it has either passed around the nucleus to form the acrosome, or migrated back along the axial filament. I agree with Tönniges that this structure, although in many stages similar to the Nebenkern of insects, is not homologous with it. In Scolopendra, however, the fibres — or later, the membrane formed by the union of the fibres — do not attach themselves

to the axial filament, as he has described for Lithobius. In some stages appearances which might lead to such an opinion are occasionally met with, but in the great majority of cells there is at no time a condition which would warrant this conclusion. In early stages each of the fibres ends free in the cytoplasm, but later they become united to form a membrane, which likewise ends free in the cytoplasm without having any connection with the axial filament.

The similarity of this differentiation of the archoplasm to the "Faserkorb" of Meves cannot be doubted. The structure has nearly the same history in Scolopendra as in the guinea pig. In both it first appears in the form of fibres. Later these fuse to constitute a membrane extending backward from the nucleus. For a time this membrane grows in length, and then it gradually disappears. I think it is possible that this structure may aid in the formation of the tail membranes, as suggested by von Lenhossék ('98) and Niesing (:00), but I have not been able to find any trace of it in the mature spermatozoön.

### C. *Axial Filament.*

The origin of the axial filament in the spermatids of myriapods has been explained in one way by Tönniges (:02), in another by Meves (:02[b]). According to the account of Tönniges the filament in Lithobius arises in very much the same manner as here described for Scolopendra. It appears during the course of the migration of the centrosomes from the periphery of the cell toward the nucleus. Although it always arises in close relation to the distal centrosome, Tönniges contends (by implication at least) that it is formed, not from centrosome substance by an elongation of one of these bodies, but rather by a metamorphosis of the cytoplasm.

Meves (:02[b], p. 501), on the contrary, from a study of the same animal concludes, "dass der intracellulare Faden (Achsenfaden, Tönniges) von Centralkörpersubstanz gebildet wird." In the early stages two centrosomes (Boveri's centrioles) lie with their axis perpendicular to the cell membrane. "Beide Centralkörper rücken dann auf den Kern zu. Dabei bleibt der distale durch einen Faden, der aus Centralkörpersubstanz ausgesponnen wird, mit der Zellperipherie in Verbindung."

From preparations of Lithobius which I possess, I can confirm the observations of Tönniges. For in Lithobius mordax, as well as in Scolopendra, the axial filament arises during the migration of the centrosomes away from the cell wall but is not connected with the cell membrane.

Concerning the process in Geophilus, P. et M. Bouin state in a brief paper (:03) that the axial filament is not formed from the substance of the distal centrosome, as "ce dernier se distingue toujours avec son congénère, au niveau de son extrémité." The results of Collin (:01) upon the same material are similar in this respect.

From these results and my own on Scolopendra heros, I believe it is safe to conclude that the axial filament is not formed from centrosome substance, but arises in the vicinity of the centrosome as a differentiation of the archoplasmic portion of the cytoplasm. It has been noted that, during the early stages of metamorphosis, when the axial filament is still enclosed in the mass of archoplasm, it is immediately surrounded by a mantle of this substance, which shows its differentiation from the rest of the archoplasm by its less granular and more transparent appearance. I have observed in Lithobius about the same condition in much more advanced cells, in which the axial filament has attained a considerable length; but in Lithobius it is apparently the cytoplasm which shows this differentiation. This, however, I do not consider as a fundamental difference, for, as we have seen, the archoplasm in the resting cells of Scolopendra is but slightly differentiated from the ordinary cytoplasm. Further evidence, though indirect, of the cytoplasmic origin of the axial filament is furnished by those peculiar formations arising in the cytoplasm of the lengthening spermatid which I have described under the name of pseudo-axial filaments. These are similar in structure and staining reaction to the true axial filament in its early stages; but there can be no doubt that these are formed from the cytoplasm or the disseminated archoplasm. They are entirely distinct in their origin from the centrosomes, which certainly contribute nothing to their formation.

The migration of portions of archoplasm along the axial filament I consider as additional evidence pointing toward the archoplasmic origin of the filament. In insects the Nebenkern has often been figured as breaking up into smaller portions which migrate along the filament. In material in which this has been described, however, the cells were usually so small that the fate of these structures could not be followed as clearly as is possible in the large spermatids of Scolopendra. Here the migration of the archoplasm occurs just at the time when the cell and axial filament are lengthening very rapidly, therefore at a time when this material can be used in building up this filament. As the axial filament continues to lengthen, these masses become smaller and fainter, and finally disappear entirely.

The results of investigations upon other groups of animals are too well known from recent reviews to make it necessary to repeat them here. It should, however, be stated that Suzuki ('98) in elasmobranchs and von Korff ('99) in Helix have shown that the proximal portion of the axial filament arises merely as an elongation of one of the centrosomes. But in the vast majority of animals this has been proved to be not true.

### D. *Centrosomes.*

The behavior of the centrosomes during the spermatid transformations is so very different in different animals that I will mention in this connection only observations made upon chilopods.

On Geophilus the observations of Collin ('01) and of P. et M. Bouin ('03) working in the same laboratory are quite at variance concerning the behavior of the centrosomes. Collin sees the centrosome first upon the nuclear membrane, and from this the axial filament runs out. At the close of the second spermatocyte division in Geophilus linearis, according to the observations of P. et M. Bouin, the centrosome lies upon the inner face of the cell membrane. The "centrosome" contains two closely united "centrioles," which are oriented perpendicularly to the cell membrane. The "centrosome" is a mass of cytoplasmic substance around the "centrioles." The nucleus moves toward the "centrosome" and comes into contact with it. "On voit alors apparaitre sur le centriole le plus externe ou distal une très mince expansion. Celle-ci s'étend entre le centriole distal et la membrane cellulaire." The nucleus and centrioles now move toward the centre of the cell. "Ce premier rudiment du filament axile ne provient donc pas de l'allongement du centriole distal, puisque ce dernier se distingue toujours avec son congénère, au niveau de son extrémité." When the axial filament has attained a considerable size and the cell has begun to lengthen, "Le centriole proximal pénètre dans le noyau et fait saillie à la surface interne de la membrane nucléaire. Puis il grossit peu à peu et prend une taille gigantesque." It remains connected with the external "centriole" by means of "un pédicule plus ou moins long et grêle." The nucleolus sometimes applies itself to the mass, which it seems to augment, but it never fuses with the mass. The body thus formed is gradually dissolved, but a delicate rod connecting with the distal "centriole" still remains. The distal "centriole" "se dédouble ensuite et les granules-filles s'écartent légèrement l'un de l'autre."

The account of the brothers Bouin is very brief and being unaccom-

panied by figures, it is difficult to compare the phenomena observed by them with those occurring in Scolopendra. Yet their recognition of the centrosome within the nucleus seems significant. It would appear likely that a further study of Geophilus might reveal a condition more nearly approaching that in my material. From their description, and in the absence of figures, it seems possible that the double "centrioles" described may correspond to the centrosomes within the nucleus of the mature spermatozoa of Scolopendra.

It is much more difficult to correlate the observations of Tönniges upon Lithobius with the processes which I have observed in Scolopendra. After the completion of the second spermatocyte division the centrosome lies upon the nucleus. Soon, however, it "verlässt seine Stellung am Kern und rückt an die Peripherie der Zelle. Dicht an diese angedrückt findet es sich als ein kleiner, sehr dunkel gefärbter, zweitheiliger Körper, dessen beide Hälften durch eine hellere Substanzbrücke verbunden sind. . . . Sie rücken bald wieder von der Peripherie ab, nehmen aber von ihr einen dünnen Faden, den Axenfaden mit, der sich zuerst ganz dicht an sie anschmiegte und erst allmälig mit dem Weiterwandern der Centrosome von ihr ablöste." As the centrosome approaches the nucleus, the nuclear membrane is drawn out into a protuberance, whereas later, when it comes to rest upon the nucleus, it lies in a small depression. In several of his figures, however, the centrosome seems to lie entirely inside the nuclear membrane. Soon the archoplasm in the region of the centrosomes gives rise to the Faserkorb mentioned above, and both cell and nucleus continue to elongate. "Innerhalb des Faserkorbs vollzieht sich die weitere Umwandlung der Centrosome, und zwar besteht dieselbe zunächst darin, dass sich der distale Theil ablöst und in das Cytoplasma vorrückt, eine Zeit lang noch durch einen Faden mit dem anderen in Verbindung bleibend; später legt er sich dem Axenfaden an und bildet einen Ring, der ziemlich nahe am proximalen Centrosoma liegt, welches letztere zum Endknöpfchen geworden ist." The section lying between the two centrosomes Tönniges interprets as the middle piece.

The observation of Tönniges in regard to the centrosomes which is most at variance with those on Scolopendra is the one concerning the transformation of the distal centrosome into a ring surrounding the axial filament. There are most certainly no appearances in Scolopendra which could lead to such a conclusion, and it may be added that the figures of this process given by Tönniges are not very convincing, for his "rings" have the appearance of mere thickenings of the axial filament caused by

the backward migration of the distal centrosome. In that case this body would correspond to the extra-nuclear centrosome in Scolopendra.

### E. *Acrosome.*

In recent years the origin of the body forming the apex or perforating part of the spermatozoön — named by von Lenhossék ('98) the acrosome — has been made out in a number of animals representing diverse groups. It has been shown that in many if not all cases it arises from some portion of the archoplasm. In insects Platner ('89) describes the acrosome as being formed from the centrosome. The later works by Henking ('91), Wilcox ('96), Paulmier ('99), and Meves (:00), however, show conclusively, I think, that Platner was in error, and that the acrosome arises either from a constricted-off portion of the Nebenkern or from a structure coexistent with this and having a similar origin from some portion of the archoplasm.

The results in the case of vertebrate material have been quite similar. In the elasmobranchs Moore ('95) has ascribed an archoplasmic origin to the acrosome; while in the Amphibia, Meves ('97) and McGregor ('99) respectively conclude that it is formed either from the entire idiosome or from a portion thereof. Niessing (:96) states that in mammals the centrosome is contained in the acrosome; but that this conclusion is erroneous is shown by the later work of von Lenhossék ('98) and Meves ('98, '99). Von Lenhossék derives the acrosome from the cytoplasm, while Meves shows that both in the rat and guinea pig it is produced directly by a metamorphosis of the idiosome.

Concerning the formation of the acrosome in Lithobius, Tönniges says, "Die Sphäre, welche ursprünglich mit den Centrosomen in Verbindung stand und sodann den Zusammenhang mit ihnen verlor, legt sich dem Kern an und bleibt nunmehr dicht an ihm liegen." A vacuole appears within it, when the axial filament unites with the nucleus, and later its substance is condensed into a deeply staining corpuscle, the apical piece, "welches mit der Spitze des zum Kopf gewordenen Kerns mittelst eines kurzen Stiels in Verbindung steht." In connection with this corpuscle there appears a deeply staining substance which covers the anterior end of the nucleus in the form of a thin cap. From the figures of Tönniges it appears to me that the body seen at one side of the nucleus at early stages, which he has interpreted as the sphere, in reality corresponds to the extruded karolymph observed in the corresponding stage of Scolopendra. (Compare my Figures 80, 81, with Figure 316, A, of Tönniges.)

The appearances are very much alike in both cases, and in each this stage is followed by an apparent growth of the nucleus. However, I believe that the acrosome is in reality derived from the archoplasm, as he concludes; but I do not believe the structure described as the sphere in his Figure 315, A, is such. At least in Scolopendra the filaments of the acrosome appear much later.

Collin and P. et M. Bouin do not trace the origin of the acrosome, but describe its appearance at the anterior end of the nucleus at the time when this is beginning to elongate. It is improbable that Geophilus differs from other chilopods in this matter.

Perhaps the most remarkable fact in regard to the acrosome in Scolopendra is its immense size as compared with the similar structure in other animals. It is indeed surprising that there should be such a difference in this regard between Scolopendra and animals so closely related to it as Lithobius and Geophilus. Yet from my own observations upon the spermatids of these forms, I am certain that such differences exist. I have studied only maturing spermatozoa as they occur in the still developing testis, and it is possible that they may mature still further and lose at least a portion of the acrosome. Yet this would not explain the differences existing in an earlier stage, for at all times the acrosome in Scolopendra is large and conspicuous, whereas in Lithobius it is always small. It is possible that some structural condition may exist in the eggs of Scolopendra which necessitates the enormous development of the acrosome.

## V. Summary.

After the last spermatogonial mitosis the small cells resulting enter upon a period of extraordinary growth, by which their diameter is increased from five to ten times. *These growing cells are early divisible as regards size into two classes.* Those of the larger type remain united in pairs during the growth period by means of the persisting interzonal filaments, *and lie in uncrowded regions of the testis, where they are surrounded by a plentiful supply of food material.* The cells of the smaller type do not remain thus united, and *are so crowded that a rich supply of nutriment is not possible.*

At the completion of the growth period — "the vesicle stage" — the spermatocytes bear a strong resemblance to egg cells during the germinative-vesicle stage, and *this is especially true of the larger type of cells, which have been surrounded by conditions similar to those of the growing egg.*

Chromatin. — Early in the telophase of the last spermatogonium all but one (the accessory chromosome) of the thirty-three small chromosomes lengthen out into granular filaments and by their union in pairs cause a pseudo-reduction of chromosomes. Upon the reconstruction of the nuclear membrane the chromatin filaments become distributed throughout the nucleus, and it is seen that the *pseudo-reduction is accomplished by an end to end union of entire chromosomes during the telophase of the last spermatogonium.*

Meanwhile the accessory chromosome has retained its homogeneous appearance and is still a simple univalent chromosome.

With the re-establishing of the nucleus changes begin which result in all the *granular chromatin segments becoming massed around the accessory chromosome to form the karyosphere.* The karyosphere is not a homogeneous body of chromatin, but a dense mass of chromatin filaments, in which identity of chromosomes is doubtless preserved.

In the following prophase the *chromosomes arise from the karyosphere by a mere unwinding of the component chromatin filaments.* These, as they become free, quickly undergo a longitudinal and later a transverse division, thus forming the cruciform tetrads typical of arthropods. Modifications of this form, such as rings and double V's, are common.

The accessory chromosome, still homogeneous in appearance, shows evidence of only a single division — the longitudinal one.

During the first spermatocyte division the *sixteen ordinary chromosomes undergo longitudinal fission, while the accessory chromosome passes into one cell undivided.*

The prophase of the second spermatocyte is of very brief duration, and the following separation of the chromatids of the ordinary chromosomes occurs along the plane of *transverse division.* But the accessory chromosome, when present, divides *longitudinally.*

Of the *four spermatids derived from each primary spermatocyte two possess an accessory chromosome, while the other two do not.*

Centrosome and Archoplasm. — The archoplasm derived by the breaking down of the spindle of the last spermatogonial mitosis persists during the growth-period and increases until in the vesicle stage a conspicuous mantle of reticular archoplasm surrounds the nucleus.

During the entire growth period *the centrosome persists* as two small dark bodies surrounded by a specialized portion of the archoplasm — the idiosome — resulting from the breaking down of the proximal portion of the astral rays and spindle fibres of the last spermatogonial division.

*Large Spermatocytes.* — The first change during the prophase of the

first spermatocyte consists in the disappearance of the mantle of archoplasm, which *becomes dissolved and in this condition distributed throughout the hyaloplasm of the cell*, the presence of archoplasmic substance being shown by the darker stain of the inter-reticular areas.

The centrosome moves to the nucleus, and divides; the resulting centrosomes separate, migrating along the membrane. During this separation astral rays arise, and by the time the centrosomes are 180° apart have become very numerous and distinct.

The spindle of the large spermatocytes, when first formed, *has its axis perpendicular to the long axis of the cell.* During the metaphase it rotates, and finally, in the early anaphase, comes to lie with its axis *lengthwise of the cell.*

During this rotation of the spindle the astral rays increase both in number and length, and the hyaloplasmic areas resume step by step their transparent appearance. *The astral rays are believed to be formed by the direct transformation of the latent archoplasm, as distributed in the hyaloplasm, into the kinetic fibrillar form.*

In the telophase the persisting spindle remnants are often detached from both cells during the rotation of the cells. The rest of the archoplasm is again dissolved and distributed through the hyaloplasm. In the following prophase the astral systems again arise by a transformation of this diffused archoplasm.

During the prophase the *centrosomes move apart along the cell membrane*, and in all subsequent stages of the second spermatocyte division retain this peripheral position.

*Small Spermatocytes.* — During the early prophase of the first mitosis the archoplasm collects in a mass upon one side of the nucleus. As the centrosomes diverge and the astral systems appear, this mass of archoplasm gradually disintegrates.

When the centrosomes are about 100° apart the nuclear membrane breaks down and an unsymmetrical spindle is formed. This soon elongates to form a symmetrical well-developed spindle.

In the late metaphase the *centrosomes leave the poles of the spindle and migrate toward the cell wall. The spindle fibres still converge toward the apical points*, — the points formerly occupied by the centrosomes, — while *the astral fibres radiate from the centrosome.* The apical point and centrosome remain connected by a few sharply defined fibres.

*The second spermatocyte division* is the same in both the large and the small types of cells.

The phenomena connected with the division of both types of sperma-

tocytes support in many particulars the old hypothesis of division by fibrillar contraction.

In both types of spermatocyte *the centrosome is the small, inner, deeply staining body at the centre* of the aster. The larger more diffusely staining structure surrounding this — *the centrosphere — varies greatly in size and appearance* in different stages of mitosis, and shows at various times that *it is merely a reserve supply of archoplasm,* which is used as occasion demands in the formation of the astral rays.

Metamorphosis of the spermatids. — The spermatids derived from *the two types of spermatocytes produce spermatozoa differing in no observable particular* except that of size. They are probably all functional.

No true Nebenkern exists in Scolopendra, as the remnants of the spindle are cast out of the cell in the telophase of the preceding division. In later stages of metamorphosis there arises from a portion of the archoplasm a structure which is comparable to the Faserkorb found in the spermatogenesis of mammals.

In the process of lengthening, the spermatid first assumes an amoeboid appearance, and later elongates in the direction of least resistance.

*The axial filament arises in close relation with the centrosome, but not from centrosome substance. There is considerable evidence pointing toward its origin from archoplasm.*

Soon after the appearance of the first fundament of the axial filament, *the centrosome breaks up, usually into three parts.* One of these retains its positions at the proximal end of the filament, whereas the others take up positions at equal distances from the first and upon opposite sides of it.

After the cell has begun to lengthen *the lateral centrosomes enter the nucleus, where they come to be connected with the median centrosome by means of fibres.* This relation is retained throughout all later stages of metamorphosis and in the mature spermatazoön.

The acrosome arises from a number of small spherules of archoplasm, which become vesicular and soon fuse to form a large club-shaped vesicle ; this comes to lie with its narrower end in contact with the anterior end of the nucleus. In later stages of metamorphosis it lengthens out into a long, slender pointed filament.

The chromatin of the spermatid undergoes a chemical change whereby it loses its affinity for chromatin stains. Later, in the spermatozoön, the chromatin reappears in an apparently amorphous condition, being deposited at the periphery of the elongated head.

In the process of elongation the nucleus first becomes dart-shaped; later it lengthens out into a very slender filament, which has upon its periphery a spiral ridge of chromatin.

The mature spermatozoa derived from the large spermatids reach a length of nearly one millimetre, the head and acrosome being 260 micra long.

Zoölogical Laboratory, Harvard University,
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## EXPLANATION OF PLATES.

All drawings were made with the aid of a camera lucida. In the description of each figure the final magnification is given. For Figure 1 a Bausch and Lomb $\frac{2}{3}$-inch objective and one-inch ocular were used, giving a magnification of 188 diameters. This was reduced $\frac{1}{5}$ in reproduction. Where a final magnification of 960 diameters is recorded it was produced by reducing $\frac{1}{2}$ from drawings made with a Leitz $\frac{1}{12}$-inch objective and a Zeiss No. 8 ocular. A final magnification of 1280 diameters was produced by a $\frac{1}{3}$ reduction from drawings made in the same manner. Final magnifications of 1310 and 1747 diameters were produced by a $\frac{1}{2}$ and $\frac{1}{3}$ reduction, respectively, of drawings made with a Zeiss $\frac{1}{18}$-inch objective and a Zeiss No. 8 ocular. Figures having a final magnification of 900 diameters are reduced $\frac{1}{2}$ from drawings made with a Bausch and Lomb $\frac{1}{12}$-inch objective and $\frac{3}{4}$-inch ocular.

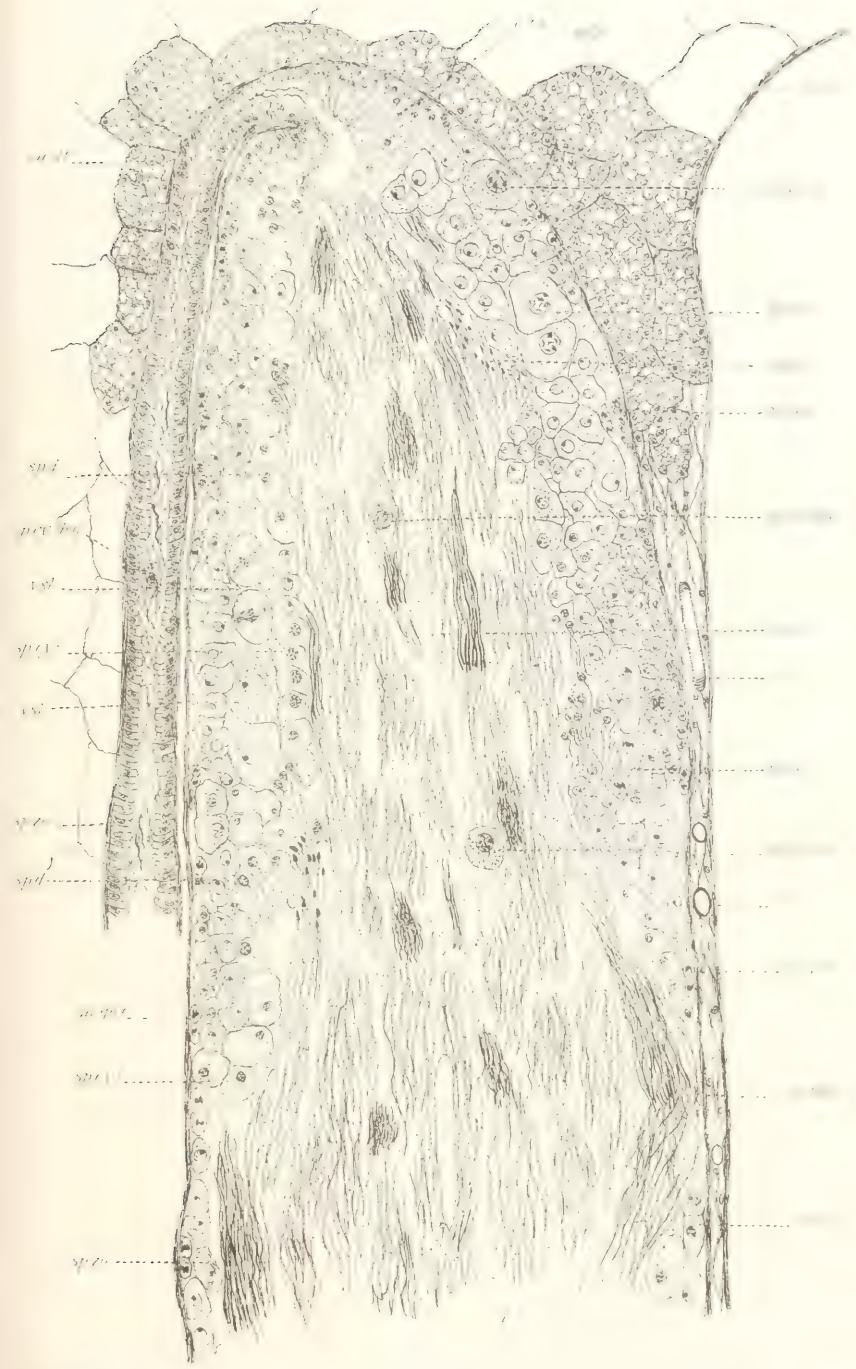
All figures are made from preparations of *Scolopendra heros* except Figure 132, which is of *S. subspinipes*.

### Abbreviations.

| | |
|---|---|
| *adp.* | adiopose tissue. |
| *e'th.* | epithelium. |
| *chr'so. acc.* | accessory chromosome. |
| *sp'cy.* | spermatocyte in growth period. |
| *sp'cy* 1. | prophase of spermatocyte. |
| *sp'cy* 2. | metaphase of spermatocyte. |
| *sp'cy* 3. | anaphase of spermatocyte. |
| *sp'cy. deg.* | degenerating spermatocyte. |
| *sp'cy* II. | second spermatocyte. |
| *sp'd.* | spermatid. |
| *sp'go.* | spermatogonia. |
| *sp'go* 4. | telophase of spermatogonia. |
| *sp'zo.* | spermatozoa. |
| *tr.* | trachea. |
| *tu. foll.* | follicular sheath. |
| *va. df.* | vas deferens. |
| *vsl.* | "vesicle" stage. |
| *vsl'.* | "vesicle" stage of large spermatocytes. |

## PLATE 1.

FIG. 1. Section of the proximal end of a follicle of the testis of *Scolopendra heros* where it connects with the vas deferens, showing the general arrangement of the various cell generations. × 150.



## PLATE 2.

*All figures magnified 960 diameters.*

FIG. 2. Resting stage of *spermatogonium* of Scolopendra heros. All of the chromatin is massed in one body, the karyosphere. The cell membrane is much less distinct than the nuclear membrane.

FIG. 3. Prophase of a spermatogonium. Chromatin in the form of 33 small chromosomes. All of these except one, the accessory chromosome, are granular. Centrosomes are now to be seen.

FIGS. 4, 5. Metaphase and anaphase, respectively, of the last spermatogonial division.

FIG. 6. Late anaphase of spermatogonium, as seen in a section oblique to the axis of the spindle.

FIG. 7. Telophase of last spermatogonial mitosis. The ordinary chromosomes have begun to lengthen and become granular, while the accessory chromosome still remains homogeneous. Centrosomes still in polar region of the cell.

FIG. 8. Synapsis. The chromatin segments have lengthened still more. Accessory chromosome unchanged.

FIGS. 9, 10. Synapsis completed. The chromatin segments, of the reduced number, are distributed more evenly throughout the nucleus, which is now enclosed in a nuclear membrane. Centrosomes have migrated from the poles.

FIG. 11. Slightly later stage, showing the beginning of the formation of the karyosphere. Many of the chromosomes show evidence that they originated by an end to end union of spermatogonial elements.

FIGS. 12–15. Later stages in the production of the karyosphere and in the growth of the young *spermatocyte*. The centrosomes are still to be seen. The remnants of the spindle of the last division still persist, and are continuous with the mantle of archoplasm surrounding the nucleus.

Figures 16-40 illustrate conditions of the *first* (*primary*) *spermatocytes* of the *large type*.

FIG. 16. "Vesicle" stage of the large spermatocytes. All of the chromatin is collected into the karyosphere, while the rest of the nucleus is less dense than the cytoplasm. A mantle of archoplasm surrounds the nucleus, and in a denser portion of this two centrosomes are visible. The persisting remnants of the spindle are still seen.

FIG. 17. Very early prophase of the large spermatocyte. The archoplasmic mantle has disappeared, and the character of the cytoplasm has altered. The centrosomes have moved to the cell membrane, and are now migrating apart along its surface.

FIG. 18. Nucleus of a spermatocyte of Scolopendra heros, showing a karyosphere similar to those characteristic of Scolopendra subspinipes.

PLATE **2.** — *Continued.*

Fig. 19. Various appearances presented by the karyosphere: *a*, in preparations not sufficiently decolorized; *b*, where the chromatin threads have been massed together irregularly by the fixing fluid; *c*, section through one side of the karyosphere; *d*, *e*, thin, well-stained sections showing the true spireme character.

Figs. 20–22. Early prophases showing the behavior of the centrosomes. In Figure 22 is shown a section through one side of the karyosphere.

Figs. 23, 24. Nuclei in later stages, showing the origin of the chromosomes from the karyosphere. A number of chromatic segments have already become detached and have undergone the early processes of tetrad formation, while others are still connected with the karyosphere.

Fig. 25. Later stage. The last chromosomes are now leaving the karyosphere, so that nothing of this remains except the accessory chromosome.

Figs. 26, 27. Nuclei showing the tetrads derived by a longitudinal and a cross division of the chromatin segment. The karyosphere is much reduced in size, but still contains several chromosomes. In Figure 26 the accessory chromosome is readily distinguished.

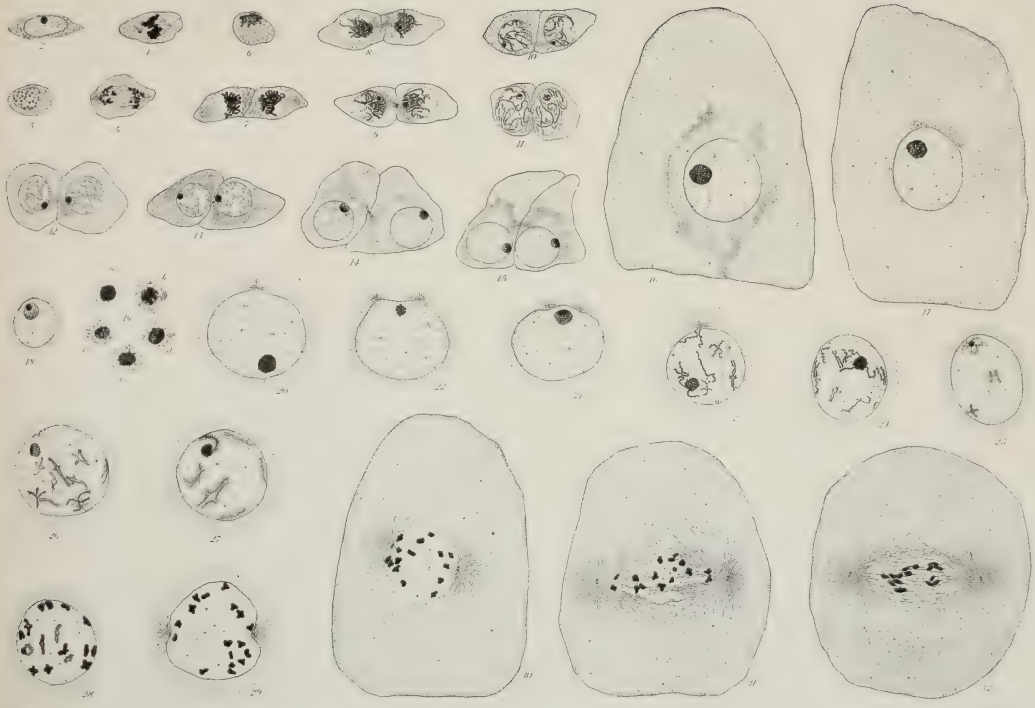
Fig. 28. The tetrads are becoming condensed, but many still show the two divisions.

Fig. 29. Late prophase. The chromosomes have become still more condensed, and are now homogeneous. The accessory chromosome is distinguishable from the others by its shape. The centrosomes are at opposite sides of the nucleus.

Fig. 30. Still later prophase. The nuclear membrane is breaking down.

Fig. 31. Very late prophase. The nuclear membrane has disappeared and the spindle is being formed.

Fig. 32. Metaphase. The cell outline has become oval, and the spindle still extends across the shorter diameter of the cell. The cytoplasm in the region of the astral system is more open than in the peripheral region.



## PLATE 3.

*All figures magnified 960 diameters.*

FIG. 33. Later metaphase. The spindle has begun its revolution. The centrosomes have completely divided, and the centrosphere is partially constricted.

FIG. 34. Later stage in the revolution of the spindle. Nearly all of the cytoplasm now appears transparent.

FIG. 35. Early anaphase. The revolution of the spindle is completed and the chromosomes are being separated. The astral radiations now extend to all portions of the cell.

FIG. 36. Late anaphase. The chromosomes have nearly reached the poles. In one group there are seventeen chromosomes, while in the other there are only sixteen.

FIG. 37. Slightly later anaphase, showing the beginning of the constriction of the cell wall.

FIG. 38. Early telophase of the first spermatocyte. The constriction of the cell is nearly completed. The "Zwischenkörperchen" is very well developed. The chromosomes lie in an irregular clear vesicle.

FIG. 39. Late telophase. The constriction of the cell is complete, and a portion of the "Zwischenkörperchen" lies in neither cell. The nuclear membrane is re-formed, and the chromosomes, with the exception of the accessory chromosome, have become granular.

FIG. 40. Four drawings showing the shape and size of the chromosomes and the relation they bear to the spindle fibres. In each drawing there is one chromosome which shows a notching at the ends, and is connected with only one pole by mantle fibres. This is the accessory chromosome, *chr'so. acc.*

Figures 41–46 represent consecutive stages of *secondary spermatocytes.*

FIG. 41. Two cells derived from one primary spermatocyte, each in the early prophase of the second division. The ordinary chromosomes all show a dumbbell form, while in one cell (the lower) the accessory chromosome is recognizable by its notched ends. The centrosomes are moving apart along the cell membrane. The cells have so rotated that the remnants of the spindle have been detached.

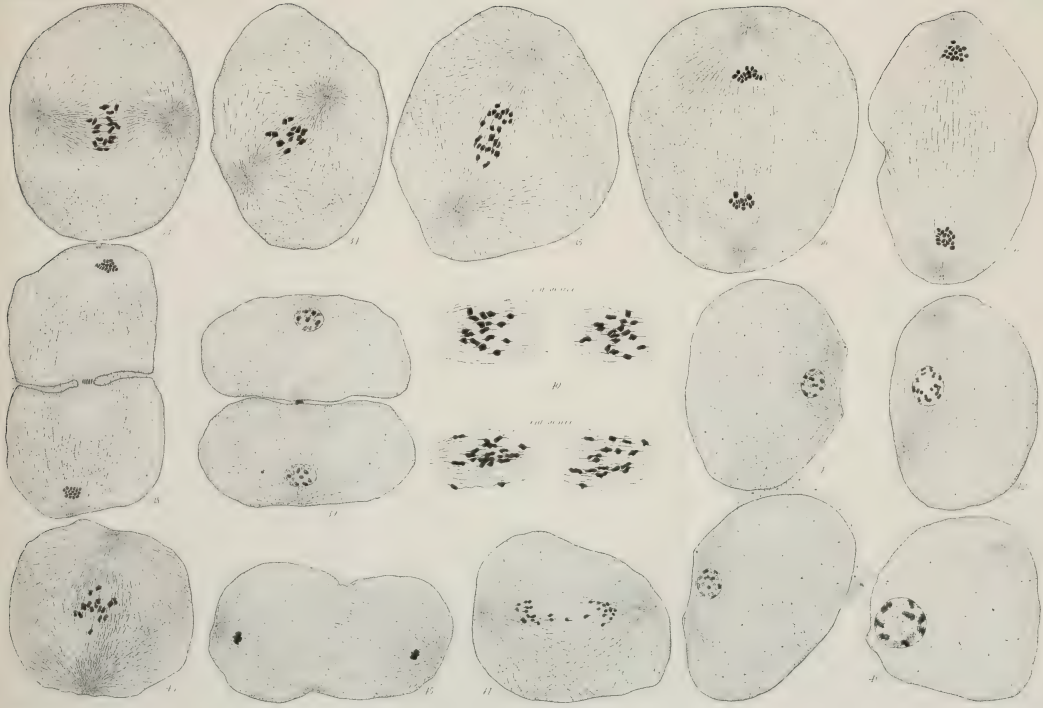
FIG. 42. Later prophase of the second spermatocyte. Each of the centrosomes is now surrounded by a centrosphere.

FIG. 43. Metaphase of the second spermatocyte. The accessory chromosome is distinguishable from the others by its size and shape.

FIG. 44. Anaphase of the second spermatocyte.

FIG. 45. Early telophase. The chromatin is enclosed in a clear vesicle. The cell wall is being constricted.

FIG. 46. Early spermatid.



## PLATE 4.

*All figures magnified 1747 diameters.*

Fig. 47. Centrosome and portion of nucleus of first spermatocyte during mid prophase. No centrosphere is present. The developing astral rays abut directly upon the centrosome.

Fig. 48. Later prophase. Nuclear membrane disintegrating. The centrosome is now surrounded by a centrosphere, through which some of the astral fibres penetrate, while the majority can be traced only to its surface.

Fig. 49. Very late prophase. The nuclear membrane has disappeared, and the linin network is being drawn toward the centrosome to form the mantle fibres. Centrosome and centrosphere are very distinct, and outside the latter another irregular darker area is formed by the closely apposed bases of the astral rays.

Figs. 50*a*, 50*b*. Pole view and side view, respectively, of the centrosome and aster in the metaphase. The centrosomes are nearly divided, but are still connected by a band of similar material.

Fig. 51. Centrosome and aster in the anaphase. The centrosomes are fully divided and quite separate from each other. The centrosphere is also nearly entirely constricted.

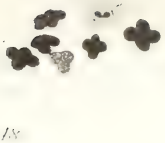
Fig. 52. Centrosome and astral rays during the separation of the centrosome in the prophase of the second spermatocyte division. No centrosphere is present.

Fig. 53. Astral system in the metaphase of the second spermatocyte. The centrosphere is again very evident.

Fig. 54. Portion of one of the small spermatocytes during the metaphase (from the same cell as Fig. 65). The centrosome is elongated in the direction of the axis of the spindle into a cone-shaped body. The centrosphere is of the same general shape. The astral rays in the polar region are fewer and fainter than those proceeding toward the equator of the cell. The latter are much branched.

Fig. 55. One of the small spermatocytes in the early telophase. No centrosphere is present, since the substance forming this in earlier stages has been used up in the lengthening of the equatorial astral rays.

47



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50a

50b

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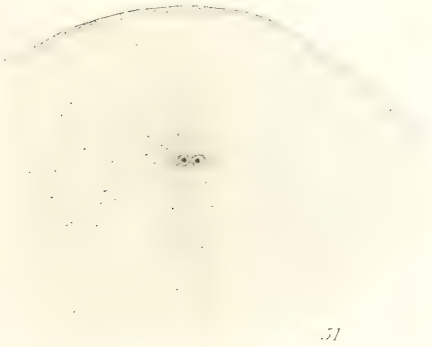

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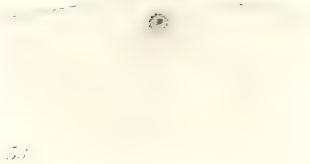

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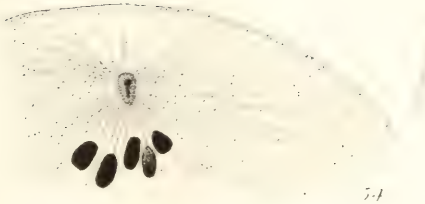

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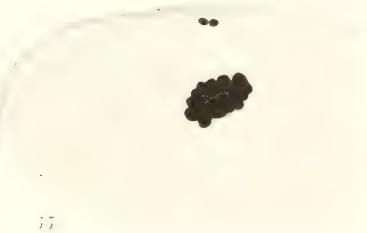

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## PLATE 5.

*All figures except 58, 67, and 71, are magnified 900 diameters.*

Figures 56–70 show the conditions in the *primary spermatocytes* of the *small type.*

Fig. 56. "Vesicle" stage. In general appearance the cell is similar to those of the large type except in size.

Fig. 57. Early prophase, showing early stages in tetrad formation. The archoplasm has collected at one side of the cell around the two centrosomes.

Fig. 58. Later stages in tetrad formation. The accessory chromosome is now plainly seen. × 960.

Fig. 59. Still later prophase. The chromosomes are becoming condensed. The centrosomes have approached the nuclear membrane and begun to separate from each other. Astral rays have arisen at the expense of the archoplasmic mass.

Fig. 60. Various stages and forms of tetrads; *a*, *b*, *c*, early, mid, and late prophase, respectively.

Fig. 61. Prophase, later than that shown in Figure 59. The centrosomes have separated still further, moving along the nuclear membrane. All of the archoplasmic mass has been converted into astral rays.

Figs. 62, 63. Late prophase. The nuclear membrane has disintegrated and the spindle is forming. In Figure 63 evidences of the formation of the mantle fibres from the linin network are seen.

Fig. 64. Still later stage, showing the extreme shortness of the spindle as first formed.

Fig. 65. Metaphase. The centrosomes and centrosphere are cone-shaped. The astral fibres are branched and also cross one another in the equatorial region.

Fig. 66. Late metaphase, showing the modified spindle. Some of the chromosomes have already divided.

Fig. 67. Anaphase. All of the chromosomes with the exception of the accessory one have divided and are passing toward the poles. This special chromosome is moving toward one pole undivided. × 960.

Figs. 68, 69. Telophase. The chromosomes are massed near their respective poles, and the individual elements are indistinguishable from one another. In Figure 69 the constriction of the cell wall has begun.

Fig. 70. Late telophase.

Figures 71–76 represent stages of the *secondary spermatocyte* of the *small type.*

Fig. 71. Prophase of the second spermatocyte. This often follows the telophase of the first spermatocyte without a reconstruction of the nucleus. The centrosomes migrate apart along the inner surface of the cell membrane as in the large spermatocytes. × 960.

Fig. 72. Metaphase.

Figs. 73, 74. Early and late telophase, respectively, of second spermatocyte.

Figs. 75, 76. Spermatids of the small type.

## PLATE 6.

*All figures magnified* 960 *diameters.*

Fig. 77. Early telophase of the second spermatocyte. The nuclei are beginning to be reconstructed. The centrosome is in close contact with the cell membrane. The cell membrane is entirely constricted except for the central portion of the remnants of the spindle, which is drawn out into a narrow connecting cord.

Fig. 78. Later telophase. The nuclear membrane has appeared. Centrosome still at periphery. The cells have drawn apart still further, causing still more of the remnants of the spindle to enter into the cord-like bridge between the two cells.

Figures 79–101 represent the *spermatid* and the early stages of its *metamorphosis* into the spermatozoön.

Fig. 79. Early spermatid. The remnants of the spindle have been detached. Centrosome still in contact with cell membrane.

Fig. 80. Early spermatid, at the time when the nucleus increases rapidly in size by the taking in of material from the cytoplasm, showing rupture of the nuclear membrane.

Figs. 81, 82. Both cells are of the same stage. A portion of the nuclear material has the form of hernia-like protrusions, extruded as result of the rapid taking in of material from the cytoplasm.

Fig. 83. Stage of about the same age as Figures 81, 82. The extruded portion has become entirely separate from the nucleus.

Fig. 84. Later stage, immediately following the enlargement of the nuclear vesicle. There is no distinct nuclear membrane. The centrosome having left the cell membrane is migrating toward the nucleus. It shows an elongation in the direction of its movement. A distinct mass of archoplasm surrounds the centrosome, and small irregular masses are to be seen at various places in the cytoplasm. The chromatin is aggregated upon the side of the nucleus nearest the centrosome.

Fig. 85. The centrosome has migrated still further, and at its distal end the beginning of the axial filament is seen. The entire structure is enclosed in a mass of archoplasm.

Figs. 86–90. Slightly later stages in the formation of the axial filament, showing the appearance of the early axial filament and the relations existing between it and the archoplasm, cytoplasm, and centrosomes.

Fig. 91. Later stage in the formation of the axial filament. The archoplasm is gathered about the base of the filament, but has already begun to break up into smaller masses. The chromatin is becoming more evenly distributed.

Figs. 92, 93. Stages showing the beginning of the elongation of the cell. The cell membrane on the side opposite the nucleus — the future posterior end of the cell — shows an irregular wavy contour. Portions of archoplasm have broken off from the main mass around the base of the axial filament, and some of these have migrated to the side of the nucleus opposite the filament, while others are moving along the course of the axial filament.

PLATE 6. — *Continued.*

Fig. 94. Slightly later stage in the process of elongation. The posterior portion of the cell shows a number of pseudopodia-like projections extending in various directions.

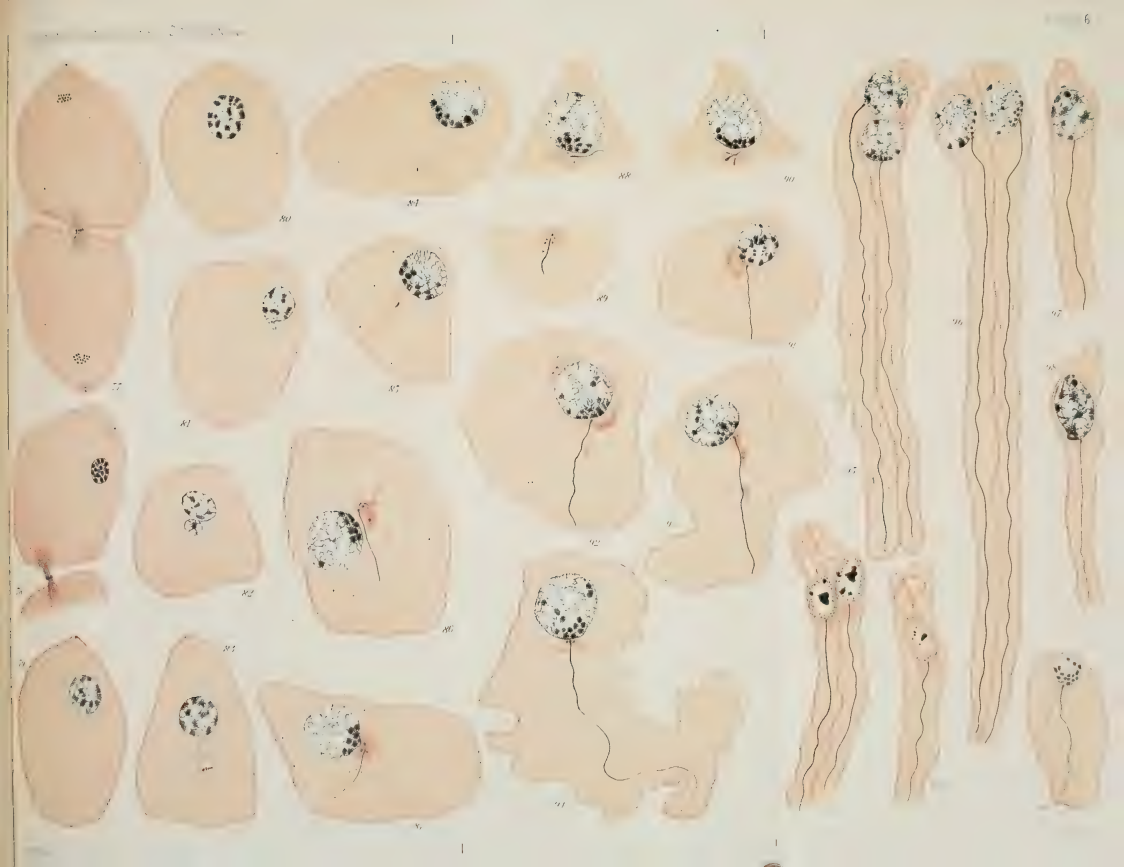
Figs. 95, 96. Later stages in the elongation of the cell and the growth of the axial filament. Archoplasmic bodies are seen at various points along the course of the axial filament. Numerous deeply staining monilated short filaments are seen in the cytoplasm. Some of the archoplasmic spherules at the anterior end of the nucleus have been converted into vesicles which are uniting (Fig. 95) to form larger ones. The chromatin bodies are being broken up into flaky masses of granules. The "lateral centrosomes" have entered the nucleus and are now indistinguishable from the chromatin granules.

Figs. 97, 98. Later stages. The acrosome vesicles are uniting to form a larger vesicle. The nucleus is beginning to elongate in the direction of the axial filament. All of the chromatin, except one dense body, the karyosphere, is deposited in diffuse flaky masses upon the linin reticulum. A small mass of archoplasm still remains around the proximal end of the axial filament.

Fig. 99. The archoplasmic vesicles have all united to form the acrosome, although portions of the partition walls still remain. The chromatin is undergoing a chemical change, by which its staining reaction is altered. The archoplasm at the base of the nucleus has become converted into diffusely stained fibres arranged parallel to the axial filament.

Fig. 100. The fusion of the acrosome vesicles is complete. In this cell the acrosome has lost its fluid contents and become flattened. The chromatin has undergone still further change.

Fig. 101. A spermatid of the small type at about the stage of Figure 94, showing the formation of false axial filaments at various places in the cytoplasm.



## PLATE 7.

*Late stages in the metamorphosis of the spermatid. Figures* 107, 108, *and* 115–120 *are magnified* 1747 *diameters: all others,* 1280 *diameters.*

Fig. 102. Later stage in the elongation of the nucleus. This is the period during which the chromatin is in its most diffuse condition, for only a very little of it stains in the ordinary manner, while the rest of the nucleus assumes a reddish gray color. At this time the two lateral centrosomes again become distinguishable. They lie in the nucleus and are connected by fibres with the end knob of the axial filament.

Fig. 103. Spermatid of the small type at about the same stage as that of Figure 102. The centrosomes are much more distinct than in the other type, Figure 102. Cells similar to this are very numerous.

Fig. 104. Spermatids slightly older than that of Figure 102. The chromatin is regaining its affinity for nuclear stains. Centrosomes still very easily distinguished. At the juncture of acrosome and nucleus a clear vacuole has appeared.

Fig. 105. Spermatid of the small type of about the same stage as that of Figure 104.

Fig. 106. The chromatin again stains deeply, and the whole of the nucleus except a small portion appears homogeneous. The acrosome has become condensed and now stains gray.

Figs. 107, 108. The nuclear portion of cells of the same stage as that shown in Figure 106 under higher magnification, showing the presence of a large vacuole at the anterior end of the head. In Figure 108 the lateral centrosomes are seen inside the head surrounded by a lighter area. The end knob appears in both figures.

Fig. 109. The nucleus is further elongated. The acrosome is also elongating, a projection being thrust out from one side of it near its anterior end.

Figs. 110–112. The nucleus and acrosome continue to elongate. At this stage numerous small vacuoles, or one large one, are often seen in the nucleus. In Figure 111 the three centrosomes are plainly seen, two within the nucleus and one forming the end knob of the axial filament.

Fig. 113. Cross sections of young spermatozoa of the stage of Figures 110–112, showing a large central vacuole in the head region. The diameter of the cell in the region of the axial filament is much less than in the head region.

Fig. 114. Later stage, showing the beginning of the spiral condition of the head.

Fig. 115. Portions of the head region of a nearly mature spermatozoön demonstrating the nature of the spiral; *a*, cross sections, showing the central clear region and the outer dark region of the head with a thickening which corresponds to the spiral ridge; *b*, longitudinal optical section, showing same structure from another point of view; *c*, surface view.

Figs. 116–119. Nearly mature spermatozoa, showing the inner lighter area of the head, the centrosomes and their relation to both head and axial filament.

Fig. 120. Portion of the head region of mature spermatoön, showing centrosomes.

Fig. 121. Entire head and axial filament of a mature spermatozoön as seen in the testis. The length of head and acrosome is 260 micra. Only one centrosome is seen, owing to the direction from which the object is viewed.

## PLATE 8.

All figures of Plates 8 and 9 are reproductions of photomicrographs made by the author. Photographs 122–165, 169, and 173 were made with the camera vertical, using daylight as the source of illumination. The Foot and Strobell (:01) method of focusing was used. Photographs 164, 165, 168, 170, and 173 were made with the camera horizontal, using a Nernst light of about 50-candle power as the source of illumination. In Figures 133, 140, 146, 148, and 154 the centrosomes have been made more prominent by retouching the negative.

*Figure* 122 *is magnified* 130 *diameters; Figure* 132, 656 *diameters; Figures* 134, 136, 139, *and* 140, 520 *diameters; all others,* 800 *diameters.*

Fig. 122. Cross section of follicle of the testis of Scolopendra heros, showing the general arrangement of the various generations of cells. × 130.

Fig. 123. Spermatogonia in the "resting stage," the same as that of Figure 2.

Fig. 124. Early prophase of spermatogonium. Two nuclei lie one above the other and so close to each other that they appear in the photograph as one.

Fig. 125. Metaphase of spermatogonium.

Fig. 126. Telophases of last spermatogonia, showing the massing of the chromatin during synapsis.

Fig. 127. Early stage of first spermatocyte at the time when the chromatin is collecting to form the karyosphere.

Fig. 128. Portions of the two spermatocytes derived from one spermatogonium. The interzonal filaments still persist.

Fig. 129. "Vesicle" stage showing a karyosphere in which the chromatin is aggregated into irregular masses.

Fig. 130. Typical "vesicle" stage of the large spermatocyte.

Fig. 131. Nucleus of same stage. The section has passed through one side of the karyosphere, showing the spireme character of the chromatin.

Fig. 132. Nucleus of first spermatocyte of *Scolopendra subspinipes*, showing the character of the karyosphere in this species.

Fig. 133. Portion of the spermatocyte in the "vesicle" stage. The two centrosomes are in the mass of archoplasm at the right of the nucleus.

Fig. 134. Late prophase. The chromatin segments have become condensed into homogeneous chromosomes. The centrosomes lie at the two poles of the nucleus, only one being shown in this section.

Fig. 135. From the same cell that is shown in the preceding figure. The second centrosome is visible in this section.

Figs. 136, 138. Mid prophase. A number of chromatin segments have arisen from the karyosphere, which has decreased in size.

Fig. 137. Very early prophase of first spermatocyte. The masses of archoplasm around the nucleus have disappeared. The centrosomes have migrated to the nucleus and begun their migration along its membrane. One is shown in the photograph, while the other is out of focus.

Fig. 139. Abnormal spermatocyte in "vesicle" stage. In such cells the chromatic material is much more plentiful than in normal cells and there may be from 2 to 10 karyospheres.

Fig. 140. Centrosome, centrosphere, and aster as seen in the early metaphase.

PLATE **8**. — *Continued.*

Fig. 141. The nucleus in the early prophase. The karyosphere is giving origin to the first chromosome.

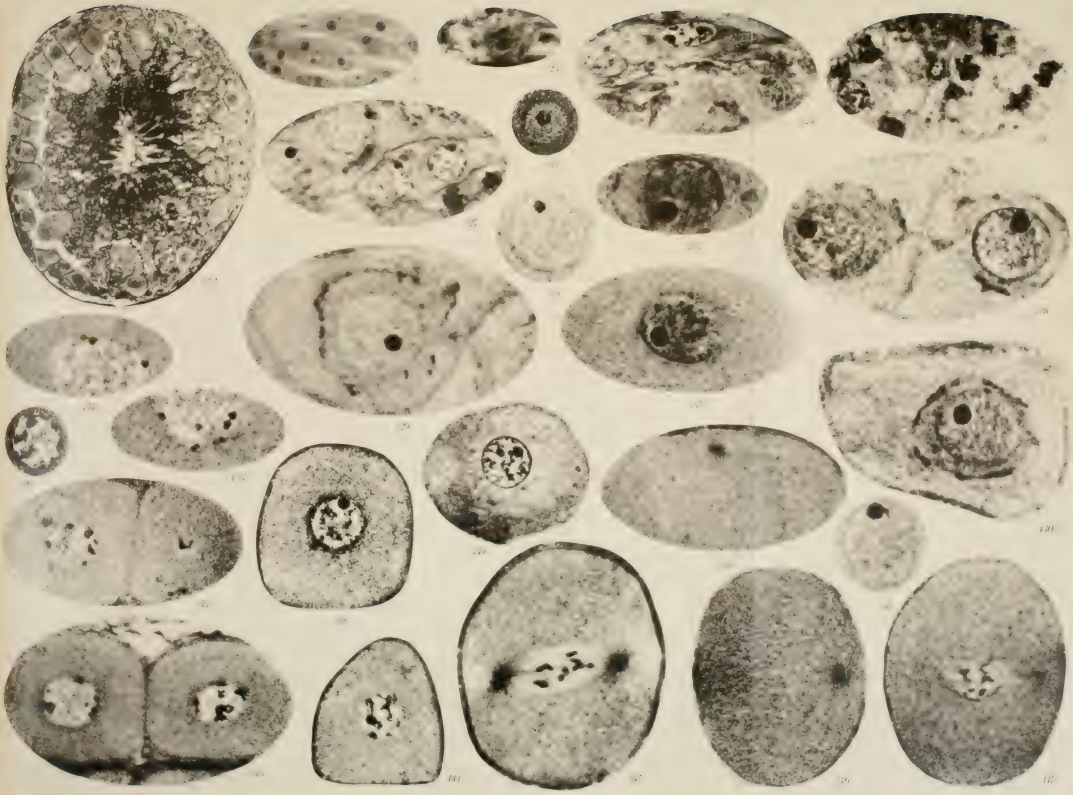
Fig. 142. Late stage in disintegration of karyosphere. The last chromosome is arising from this structure, leaving the accessory chromosome as a remnant.

Figs. 143, 144. Slightly earlier stage than that of Figure 142, showing the origin of the chromatin segments from the karyosphere, and the forms taken on by the chromosomes as they arise.

Fig. 145. Early metaphase of first spermatocyte, taken at such a focus as to give a general view of the spindle.

Fig. 146. Slightly later metaphase, showing centrosphere and centrosome.

Fig. 147. Portion of the same cell showing the spindle. The spindle has begun to undergo its rotation, and all of it does not appear in one section.



## PLATE 9.

*Figures* 153, 160, *are magnified* 520 *diameters: Figures* 164, 165, 168, 170–173, *are magnified* 656 *diameters; all others*, 800 *diameters.*

Fig. 148. Polar view of centrosome, centrosphere, and aster of spermatocyte of the large type in the anaphase.

Fig. 149. Polar view of spermatocytes of the small type in the anaphase.

Figs. 150, 151. Late prophase of second spermatocyte. In each case the centrosomes are in contact with the cell membrane, but the plane of the section is tangential to the membrane at this point.

Fig. 152. Metaphase of second spermatocyte.

Fig. 153. Anaphase of second spermatocyte.

Fig. 154. Prophase of the first spermatocyte of the small type. Chromosomes are becoming dense. Centrosomes (at left of nucleus) are migrating toward nuclear membrane.

Fig. 155. Later prophase. The centrosomes are moving apart along the nuclear membrane.

Fig. 156. Late prophase. Nuclear membrane is breaking down and spindle is being formed. (Compare Figs. 62, 63.) Only one centrosome is in focus.

Fig. 157. Short spindle of early metaphase. (Compare Fig. 64.)

Fig. 158. Later metaphase. The spindle has lengthened.

Figs. 159, 160. The modified spindle. (Compare Fig. 66.)

Fig. 161. Late anaphase of division of first spermatocyte of small type.

Fig. 162. Telophase of first mitosis, small-type spermatocyte.

Fig. 163. Metaphase of second mitosis of small-type spermatocyte.

Figs. 164, 165. Metaphase of second division of spermatocyte of large type, to show different appearance of accessory and ordinary chromosomes.

Fig. 166. Early stage of spermatid.

Fig. 167. Later stage. (Compare Figs. 95, 96.)

Fig. 168. Late spermatid (compare Fig. 103), showing one of the lateral centrosomes. The other one is out of focus.

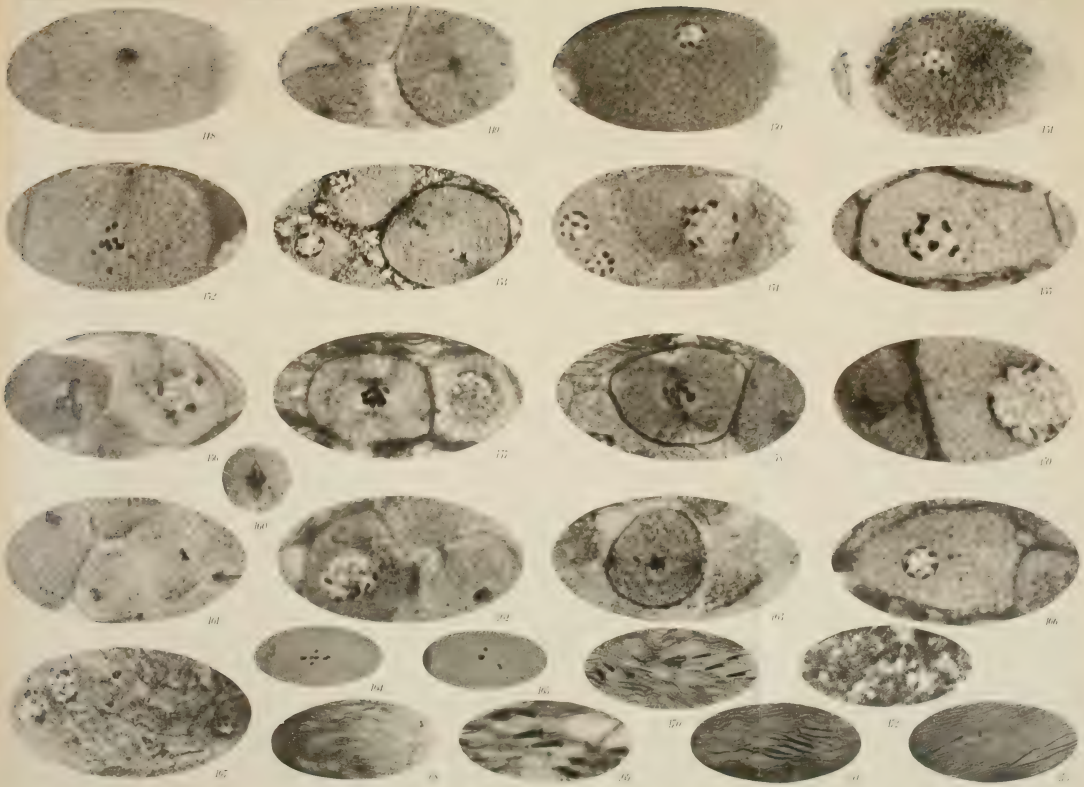
Fig. 169. Young spermatozoa (compare Fig. 106), showing head, acrosome, and axial filament.

Fig. 170. Spermatozoa of same stage as Figure 109. In those at the left the presence of the centrosome is shown by the darker mass at the base of the head.

Fig. 171. Later stage, showing head and portion of acrosome (compare Fig. 111). In the third one from the top the extra-nuclear centrosome appears.

Fig. 172. Portion of follicle in cross section, showing the appearance of the mature or nearly mature spermatozoa. The cytoplasmic sheath, even in the head region, is quite pronounced.

Fig. 173. Portions of mature spermatozoa. In the one at the left the intra-nuclear centrosomes are seen (similar to Fig. 120).



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# THE DEVELOPMENT OF THE OCULOMOTOR NERVE, THE CILIARY GANGLION, AND THE ABDUCENT NERVE IN THE CHICK.

By Frederick Walton Carpenter.

With Seven Plates.

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# *The Development of the Oculomotor Nerve, the Ciliary Ganglion, and the Abducent Nerve in the Chick.*

By Frederic Walton Carpenter.

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## Introduction.

"Von allen motorischen Nerven ist mit Ausnahme vielleicht des Hypoglossus kein anderer zum Gegenstand so widerspruchsvoller Angaben und Deutungen geworden, wie der Oculomotorius. Er ist als dorsaler, als ventraler und als gemischter Nerv in Anspruch genommen worden; man hat ihm metamerischen Werth zu- und abgesprochen; er ist als Theilstück des Trigeminus definirt, und ihm sind alle Beziehungen zum Trigeminus geleugnet worden. Man hat Ganglien an ihm entdeckt, deren Ursprungsort man in der Ganglienleiste sah; man hielt sie dann für eine Abspaltung des G. ciliare; man schrieb sie einem eigenen G. oculomotorii zu, das nichts mit dem G. ciliare zu thun habe; man leugnete die Ganglien ganz und gar — kurz es war nicht mit ihm fertig zu werden." — Dohrn ('**91**, p. 2).

Since Dohrn commented thus in 1891 upon the diversity of opinion which exists concerning the oculomotor nerve and the ciliary ganglion, three investigators have added still another to the already large number of conflicting statements. They have asserted that in selachians the oculomotor nerve grows from the mesocephalic ganglion to the ventral face of the mid-brain, and not in the opposite direction, as had previously been supposed. Dohrn himself, in the article from which the quotation is taken, described an entirely new mode of origin for the cells of the ciliary ganglion, namely, migration from the neural tube into the root of the oculomotor nerve.

This lack of agreement in regard to the developmental history of the oculomotor nerve, and particularly of the ciliary ganglion, seemed a sufficient justification for a renewed study of the subject. In the case of the abducent nerve, opinions of observers being more in accord, a study of its development might be expected to result in little more than a confirmation of generally accepted views. Though primarily concerned with the oculomotor nerve and ciliary ganglion, I have, nevertheless,

included in this paper my observations on the histogenesis of the abducens. Its study has been made easy by its presence in the series of sections in which the later development of the oculomotor has been followed, and as a typical ventral cranial nerve with motor functions it has proved interesting for the purposes of comparison with the oculomotor. The remaining eye-muscle nerve, the trochlear, first appears at a comparatively late stage, and my observations have not been extended to it.

Several considerations led to the selection of the chick as the subject for investigation. First, closely connected stages in the development of the eye-muscle nerves have been studied in but few of the Amniota, the greater part of the observations having been confined to selachians. Secondly, no investigator has directly concerned himself with the genesis of these nerves in birds since Marshall published the first accounts of their development in 1877 and 1878. Marshall's descriptions are admittedly incomplete, and certain of his interpretations are questionable in the light of more recent studies in other classes of vertebrates. Since his time observations on the nerves in question have been fragmentary and incidental. Thirdly, in the case of the chick it is possible to control incubation, and obtain embryos in the required stages of development.

It is a pleasure to acknowledge here my sense of obligation to Professor E. L. Mark, under whose guidance the present work was carried on in the Zoölogical Laboratory of Harvard University. The constant interest, the helpful suggestions and the conservative judgments of Professor Mark have been of the greatest value to me. I am also indebted to Professor H. V. Neal, of Knox College, for advice as to the use of the vom Rath fluid in the early stages of the development of nerves.

## PART I.—ANATOMY AND HISTOLOGY.

### A. Historical Survey.

#### a. Anatomy.

##### 1. *Eye-Muscle Nerves.*

The older anatomists (Muck, '15; Bonsdorff, '52; Budge, '55; and others) who first investigated the eye-muscle nerves of birds found them homologous with nerves of similar function in other classes of vertebrates. The third, or oculomotor, nerve arises from the ventral face of the mesencephalon, and is distributed to the dorsal, ventral and anterior rectus muscles and to the ventral oblique muscle. The fourth, or troch-

lear, nerve emerges from the dorsal aspect of the brain, at the posterior boundary of the mesencephalon, and innervates the dorsal oblique muscle. The sixth, or abducent, nerve takes its origin from the ventral side of the metencephalon, and passes to the posterior rectus muscle. A slender branch of this nerve is given off to the muscles of the nictitating membrane (quadratus and pyramidalis). In Bronn's Thierreich is mentioned on the authority of Bonsdorff an anastomosis between the sixth nerve and the ramus ciliaris trigemini in Corvus cornix. In this bird fibres are also said to pass to the ramus ciliaris externus of the ciliary ganglion; while in Grus cinerea a fine branch of the abducens passes partly to the ramus ciliaris internus of the ciliary ganglion, and partly to the ganglion itself. The distribution of abducent fibres to the eyeball in birds has also been recorded by Jegorow ('86–87), who considers it possible that these may be sympathetic fibres, which join with the sixth nerve as it passes through the cavernous sinus and proceed cephalad in its trunk.

### 2. *Ciliary Ganglion.*

A well-marked ganglion is always found in connection with the oculomotor nerve of birds. This ganglion corresponds to the ciliary ganglion of human anatomy, which was first described by Schacher in 1701 (Jegorow, '86–87). In man it occurs in the posterior region of the orbit as a small, laterally compressed, somewhat four-sided body, measuring about 2 mm. in an antero-posterior direction. From behind it receives branches from three different sources: a short or motor root (radix brevis) from the oculomotor nerve, a long or sensory root (radix longa) from the nasal branch of the ophthalmic division of the trigeminus, and a sympathetic root (radix sympathica) from the sympathetic plexus of the cavernous sinus. It gives off in front six to eight ciliary nerves, which proceed to the sclerotic and choroid coats, ciliary muscle, iris and cornea of the eyeball. These nerves are distinguished as the short ciliary nerves from the so-called long ciliary nerves, which emanate from the nasal branch of the trigeminus, and have the same distribution as the short ciliaries (Quain's Anatomy, Thane, '95).

The ganglion in question has received various names from different authors (ciliary, ophthalmic, lenticular, oculomotor, Schacher's). It is commonly described in the text-books of human anatomy in connection with the trigeminal nerve. It is almost invariably stated to be sympathetic in nature (as first suggested by Arnold, '31), although it differs from typical sympathetic ganglia in giving origin to medullated peripheral nerves (the short ciliaries) instead of non-medullated fibres.

Comparative studies of the anatomical connections of the ciliary ganglion have been made by Schwalbe ('79), Jegorow ('86–87), Holtzmann ('96) and Ónodi ('01).

Schwalbe, in his extensive and much-quoted work on "Das Ganglion oculomotorii," shows that the ciliary ganglion is represented in the lower vertebrates by groups of ganglion cells distributed along the course of the oculomotor nerve. Passing upward toward the higher forms, the cells become more closely associated into a compact body, this change being accompanied by a gradual withdrawal of the ganglion from the trunk of the oculomotor nerve through the formation of a radix brevis. However, not all the higher vertebrates possess short roots, since in many mammals (sheep, calf, dog, rabbit) none exists, the ganglion being placed directly on the trunk of the oculomotor. Schwalbe denies the existence of a connection between the ciliary ganglion and the trigeminal nerve in several species, and believes the sympathetic root to be confined to mammals. He, therefore, asserts that the ciliary ganglion belongs primarily to the oculomotor, which he considers entitled to the rank of an independent segmental nerve.

Jegorow's researches on "le ganglion ophthalmique" were, like those of Schwalbe, very comprehensive. As far as the anatomical relations of the ganglion are concerned, he differs from the latter writer chiefly in regard to the importance of the connection with the trigeminus. This he regards as constant, and necessary for the existence of the ganglion, throughout the vertebrate series.

Holtzmann found the ciliary ganglion in amphibians, birds and mammals more intimately connected with the oculomotor than with the trigeminus. Although he found neuraxons joining the ciliary ganglion with the fifth nerve where Schwalbe believed no connection existed, he does not regard these neuraxons, in certain cases, as constituting a physiological radix longa.

The examination of many selachians as well as several bony fishes and mammals convinced Ónodi that the connection between the ciliary ganglion and the trigeminus is more intimate than Schwalbe's researches show. In selachians he frequently found a macroscopic ciliary ganglion external to the trunk of the oculomotor. The ganglionic groups connected distally with the third and fifth nerves he considers sympathetic in nature, and in support of this view calls attention to nerve fibres extending from them to form a plexus about the wall of a neighboring blood vessel.

The observations which have already been made on the anatomical

relations of the ciliary ganglion in the group of birds will now be summarized.

*Connection with the Oculomotor Nerve.* A radix brevis is present in Strix flammea, in various species of the genus Corvus, in Falco tinnunculus and Sterna hirundo. The ciliary ganglion is placed directly on the trunk of the third nerve in the goose, Falco palumbarius, Aquila leucocephala, Meleagris gallopavo, Ardea cinerea, Vanellus cristatus and Gallinula pusilla (Gadow und Selenka, '91). To this list should be added Gallus domesticus, the pigeon and the duck (Holtzmann, '96).

*Connection with the Ophthalmic Branch of the Trigeminal Nerve.* Schwalbe ('79) describes a large ciliary nerve passing cephalad from the distal extremity of the ciliary ganglion of the goose. This nerve receives, a short distance from its origin from the ganglion, a slender ramus from the ophthalmic branch of the trigeminus, but no direct fibrous connection appears to exist between the last-named nerve and the ganglion. The same conditions were observed by Holtzmann ('96) in the hen, duck and pigeon, as well as in the goose. Schwalbe concluded from the appearances that no long root could be said to be present in birds, but Holtzmann has ascertained by microscopical examination that in the hen and goose (the only forms examined in this way) about one-fourth of the neuraxons of the communicating branch from the fifth nerve turn centrad, and, running parallel with those of the ciliary nerve, enter the ganglion. This connection he believes to be a survival of an embryonic union between the fundaments of the ciliary and Gasserian ganglia, and to possess a developmental rather than a physiological significance.

A few cases of direct connection between the fifth nerve and the ciliary ganglion have been recorded. Jegorow ('86–87) asserts that such a condition obtains in the pigeon and vulture. In Bronn's Thierreich, Muck ('15) is cited as authority for the statement that in several birds the communicating branch enters the anterior part of the ganglion. Bonsdorff ('52) describes for the crane two rami from the trigeminus which have the typical relations of long roots of the ganglion.

*Connection with the Sympathetic System.* Neither Schwalbe ('79), Holtzmann ('96) nor the older investigators discovered any evidence of a connection between the sympathetic system and the ciliary ganglion in birds. Jegorow ('86–87), while admitting that the distribution of sympathetic fibres has not been proved anatomically, infers, nevertheless, the presence of sympathetic neuraxons in the ciliary ganglion from the occurrence of certain fibres which pass from the latter to the walls of neighboring arteries. He considers it possible that sympathetic neu-

raxons may enter the third nerve in the cavernous sinus, where it comes into close relation with the cephalic extension of the cervical sympathetic system.

The only description of a sympathetic root of the avian ciliary ganglion to be found in the literature is that of Rochas ('85), who detected in the goose several fine fibres extending to the ciliary ganglion from the sympathetic plexus about the ophthalmic artery (Weber's plexus).

*Ciliary Nerves.* There is much variation in the number of ciliary nerves given off by the ciliary ganglia of different species of birds. Variations may also occur among individuals of the same species. Schwalbe ('79) states that the number may vary from one in many birds, including the hen, owl and goose, to seven in parrots.

Schwalbe figures for the goose a ciliary nerve (ramus ciliaris trigemini) emerging from the ophthalmic branch of the trigeminus distal to the origin of the communicating branch passing to the ramus ciliaris oculomotorii. Holtzmann ('96) shows that in the hen the communicating branch gives off an independent ciliary nerve to the eyeball.

### b. Histology.

#### 1. *Oculomotor Nerve.*

In birds, as well as in man (Barratt, :01) and in teleosts (Herrick, '99), both large and small medullated neuraxons are present in the oculomotor nerve. This has been shown to be the case in the pigeon by Langendorff (:00), who found the main portion of the nerve composed of neuraxons of large calibre, while smaller ones occurred near the periphery. In all forms the majority of the small neuraxons pass into the ciliary ganglion.

#### 2. *Abducent Nerve.*

I have not been able to find any description of the finer structure of the abducent nerve in birds. In man it is made up of large and small medullated neuraxons (Barratt :01).

#### 3. *Ciliary Ganglion and Short Ciliary Nerves.*

*Anatomical Evidence.* The character and connections of the cells of the ciliary ganglion of vertebrates have long been favorite topics of investigation among neurologists.

Before the silver impregnation process of Golgi had come into general use, Retzius ('81) had already demonstrated by other methods the multi-

polarity of the ciliary-ganglion cells of mammals. In a later paper (Retzius, '94, '94[a]) he confirmed his former observations by the aid of the Golgi process. Using the latter method, Kölliker ('94) and Michel ('94) obtained like results, and showed, furthermore, that the ganglion cells are surrounded by pericellular baskets of nerve fibrils. These pericellular baskets have also been demonstrated, and proved to be intracapsular, by the methylen-blue *intra-vitam* stain (Huber, '97). Inasmuch as all these conditions are characteristic of the cells of sympathetic ganglia, the investigators cited above are unanimous in declaring the mammalian ciliary ganglion to be sympathetic in nature.

On the other hand, Schwalbe ('79), a "partisan ardent," to quote Jegorow, of the cerebro-spinal character of the ciliary ganglion, found in the ciliary ganglion of the sheep and calf unipolar cells, such as are characteristic of cerebro-spinal ganglia. The crudeness of Schwalbe's methods, however, leaves his results open to question. D'Erchia ('94) discovered among the numerous multipolar cells of the cat's ciliary ganglion a few bipolar cells. Such cells were seen in both the cat and dog by Holtzmann ('96), who, furthermore, found the comparatively small ciliary ganglion of the rabbit to be composed mainly, if not wholly, of cells of the cerebro-spinal type, many being bipolar. Jegorow ('86–87) figures, in a colored plate, spinal, sympathetic and ciliary ganglion cells of the cat, prepared by Boukhaloff according to a special differential method. The cells from the spinal and ciliary ganglia present the same appearance, whereas the sympathetic cells differ in staining qualities from the others. It is interesting to compare with these figures those given by His, Jun. ('91) of the same three kinds of ganglion cells taken from an embryo cat. Cells from the ciliary and from a sympathetic ganglion closely resemble each other, being small and unipolar. Those from the vagus ganglion (which belongs to the cerebro-spinal series) are, on the contrary, larger and bipolar in character.

Haller ('98) believes that the conditions which obtain in the central nervous system of the dog-fish and trout point to the cerebro-spinal character of the representatives of ciliary-ganglion cells found in these fishes. Golgi preparations of the mid-brain show, in addition to oculomotor neuraxons proceeding centrifugally from ganglion cells in motor niduli, other oculomotor neuraxons, which have no direct connection with central ganglion cells. These neuraxons he regards as centripetal processes from ganglion cells on the oculomotor nerve (i. e., ciliary ganglion cells). Such cells are accordingly to be looked upon as homologous with spinal-ganglion cells.

A considerable amount of evidence as to the nature of the ciliary ganglion in mammals has been derived from the employment of degeneration methods. To appreciate the significance of the results obtained, it must be borne in mind that in a typical sympathetic ganglion (one of the gangliated sympathetic cord) the motor neuraxons of the white ramus ("pre-ganglionic fibres"), originating from cells within the central nervous system, pass into the sympathetic ganglia, and end in pericellular baskets of fine fibrils about the sympathetic cells. From the latter are given off, peripherally, non-medullated neuraxons ("post-ganglionic fibres"), which make up the pale sympathetic nerves. These neuraxons, together with the sympathetic ganglion cells with which they are connected, form, consequently, the terminal link in a chain of neurons.

The investigations of Bach ('96) on the rabbit show that removal of the iris and ciliary body, to which the short ciliary nerves are distributed, results in a modification of the cells of the ciliary ganglion, while those of the nidulus of the oculomotor nerve remain normal. Apolant ('96, '96') cut the oculomotor nerve of young cats near its root. The fine medullated neuraxons passing to the ciliary ganglion degenerated peripherally as far as the cells of that ganglion, while these cells, together with the short ciliary nerves, remained unaltered. Bumm ('00) confirmed Apolant's results. After injury of the intrinsic eye muscles and the nerves distributed to them, nearly all the cells of the ciliary ganglion undergo changes (Marina, '98, '99), but, as shown by the further experiments of Marina, and by those of Fritz ('99), destruction of the cornea is also followed by a slight degeneration of some of the cells of the ciliary ganglion (one-eighth of the entire number, according to Marina). From this, both writers conclude that the ganglion is a mixed one, being largely motor, but also to some extent sensory in function. Fritz ('99) infers that the ciliary ganglion is connected with the sympathetic system from the fact that changes in the cells of the ganglion occur upon extirpation of the cervical sympathetic. Bumm ('00) is also of this opinion, since the cutting of the ciliary nerves in the cat results in the atrophy of only four-fifths of the cells of the ciliary ganglion. He considers the cells affected to be those of peripheral neurons, while the cells which remain unaltered are probably connected with the sympathetic system.

The study of degeneration preparations shows, then, that at least the majority of the mammalian ciliary-ganglion cells and their processes, the short ciliary nerves, may be considered the terminal neuraxons of a

motor chain, and consequently sympathetic in their relations. The muscles innervated are of the unstriped variety.

Stefani (:01) was led to the conclusion that the short ciliary nerves have their centres, i. e., their ganglion cells, in the ciliary ganglion, by observing the effect on the cells of that ganglion when atropin is applied to the eye.

Histologically, the ciliary ganglion of birds differs from that of mammals. In the hen certain of its cells, as was first shown by Retzius ('81), are bipolar in character, each sending out two processes, which arise close together, and run either in the same or in opposite directions. Near their origins from the cell the processes are pale, but soon acquire medullary sheaths. Holtzmann ('96) examined the elements of the ciliary ganglion in the hen, duck, goose, and pigeon, finding in each of the four species both large and small ganglion cells. These were usually bipolar, but an occasional unipolar cell was observed, the single process of which soon divided into two. Holtzmann is of the opinion that while in many animals (amphibians, mammals) the ciliary ganglion contains both sympathetic and spinal cells, in birds a one-sided development, that of spinal elements, takes place. These do not, as a rule, become fully differentiated into true unipolar spinal ganglion cells, but remain in an embryonic bipolar condition.

From the foregoing, it is apparent that those cells of the ciliary ganglion of birds which have been described by investigators do not resemble histologically the sympathetic cells found in the same group. The great majority of the latter are well known to be multipolar, and, in general, to resemble the sympathetic cells of mammals (Ramon y Cajal, '91, '94; Timofeew, '98; Huber, '99).

*Physiological Evidence.* From a physiological point of view the ciliary ganglion of mammals is undoubtedly sympathetic. The experimental researches of Langley and Dickinson ('89) have demonstrated the fact that a moderate dose of nicotin, which has little, if any, effect on spinal ganglia, prevents the passage of efferent nervous impulses through sympathetic ganglia. These authors considered this result due to a paralysis of the sympathetic cells, but Huber ('97) has shown that it is more probable that the nicotin paralyzes the pericellular baskets of the preganglionic neuraxons about the cells, rather than the cells themselves. The physiological effect of nicotin has afforded, therefore, a valuable criterion for determining the character of the cells of the ciliary ganglion. After an injection of nicotin, Langley and Anderson ('92) found that the ciliary ganglion of the rabbit no longer transmitted nervous impulses.

Direct stimulation of the short ciliary nerves, however, still caused contraction of the ciliary body and the iris. The results of Langley and Anderson were confirmed for the dog and monkey by Marina ('98, '99). These investigators consequently regard it as proved that the neuraxons which innervate the sphincter iridis and the ciliary muscle are connected with the cells of the ciliary ganglion. The physiological experiments of Langendorff ('94) and Bernheimer ('97) point to the same conclusion. The former found that some time after death, when stimulation of the third nerve proximal to the ciliary ganglion was without result, excitation of the short ciliary nerves produced contraction of the iris. Bernheimer demonstrated that lesion of the oculomotor nerve in the monkey leaves the iris still active.

The physiological behavior of the ciliary ganglion of birds affords additional proof of the lack of similarity between its cells and those of the ciliary ganglion of mammals. Langendorff (:00) states, on the authority of Consiglio (:00), that, after the ciliary ganglion of birds has been subjected to the action of nicotin, stimulation of the third nerve is still followed by constriction of the pupil. He himself found that, in birds which have been bled to death, the neuraxons of the third nerve, the stimulation of which causes closure of the pupil, retained their irritability considerably longer than did those of mammals subjected to the same treatment. For these reasons he regards it improbable that an intercalation of sympathetic cells occurs, as in mammals, within the ciliary ganglion.

The fact that fibres emanating from the cervical sympathetic ganglia have no effect on the movements of the pupil in birds was established by Jegorow ('87), and has recently been confirmed by Langley (:03). In mammals, several observers have shown that stimulation of the cervical sympathetic nerve causes dilatation of the pupil (Hensen und Völkers, '68; Nawrocki und Przybylski, '91; Anderson, :03). The radially placed dilator muscle of the iris of birds is exceptionally well developed (Geberg, '83; Koganeï, '85; and others).

## B. Observations.

### a. Methods.

For the purpose of examining the main trunks of the eye-muscle nerves and their larger branches, dissections of the heads of adult fowls were made. Soon after death, the orbital cavities were opened by cutting through the conjunctiva and connective tissue, and the whole head placed in the picro-aceto-platino-osmic mixture of von Rath, the formula

for which is given on page 175. After immersion in this fluid for from three to five days, the material was carried through several changes of 70 per cent alcohol, in which the excess of picric acid was, to a large extent, removed. The head was then allowed to stand until needed in a mixture of alcohol and glycerin. It was found that this treatment, which was suggested to the writer by Mr. W. A. Willard, differentiates well the nervous from the muscular tissues, and, aided by the use of the dissecting microscope, makes possible the tracing out of the distribution of very slender bundles of neuraxons.

In preparation for the detailed study of the more important and complicated relations of the nerves under consideration another method was adopted. Certain portions of the contents of the orbit were removed entire, care being taken to avoid straining or breaking the parts, and placed for fixation in either Zenker's fluid, osmic acid or the vom Rath mixture mentioned above. After dehydrating in alcohol, and imbedding in paraffin, serial sections of suitable thickness were made. The sections were cut in planes either at right angles to, or parallel with, the axis of the main trunk of the oculomotor nerve (see Plate **1**, Figs. 1 and 2). The sections of the material fixed in Zenker's fluid were stained in acid-fuchsin. The osmic acid and vom Rath preparations needed no subsequent treatment for the differentiation of the medullated neuraxons.

Cells of the ciliary, the Gasserian (cerebro-spinal) and sympathetic ganglia were studied in the following manner, with a view to determining the number and character of their processes. The ganglia were removed from a freshly killed fowl and immersed in a 0.05 per cent solution of chromic acid, in which they were allowed to stand and slightly macerate for two or three days. They were then carefully teased apart with fine needles, and stained in acid-fuchsin. In this way a certain number of cells, retaining longer or shorter portions of their processes, were isolated from the rest of the ganglion, and prepared for examination. Other ganglia were fixed in the vom Rath mixture and studied in the form of serial sections.

### b. Anatomy.

#### 1. *Eye-Muscle Nerves and Ciliary Ganglion.*

*Oculomotor Nerve and Ciliary Ganglion.* The nidulus of the oculomotor nerve lies in the ventral portion of the mesencephalon, near the mesocoele or aqueduct of Sylvius, in relation to which it occupies a

ventro-lateral position. The situation of the nidulus in the cerebro-spinal axis corresponds to that of the somatic motor column of ganglion cells (Gaskell) of the spinal cord. The neuraxons of the cells pass ventrad to emerge from the ventral face of the mesencephalon as the third nerve, which runs ventrad and cephalad through the oculomotor foramen, and then horizontally forward (Plate **1**, Fig. 1, *n. oc'mot.*). Passing ventrad of the posterior rectus muscle, it sends a small branch (*rm. mu. rt. d.*) dorsad and cephalad to the posterior edge of the base of the dorsal rectus muscle. Just distal to this branch, a large ventral ramus (*rm. v.*) is given off, on the opposite or ventral side of the nerve trunk. This ventral ramus passes beneath the ventral rectus muscle, and runs cephalad along the floor of the orbit to terminate, as a brush of fine fibres, on the ocular face of the ventral oblique muscle, about midway of its length. A slender bundle of neuraxons (*rm. mu. rt. v.*) arises from the main trunk of the nerve in close connection with the ventral ramus, and innervates the lower face of the ventral rectus muscle, near the proximal end of the latter. From the ventral ramus, soon after it passes the anterior border of the ventral rectus muscle, a small branch (*rm. mu. rt. a.*) is given off to the adjacent edge of the anterior rectus muscle.

Immediately distal to the origin of the ventral ramus, the remainder of the neuraxons of the third nerve enter the spindle-shaped ciliary ganglion (*gn. cil.*), which measures approximately two mm. in length, and has a greatest diameter of a little less than one mm. No radix brevis can be said to exist in the hen, since it is possible, in serial sections, to trace the cells of the ciliary ganglion back as far as the level of the ventral ramus (Plate **2**, Fig. 3, *C*). From the distal end of the ciliary ganglion, a comparatively large ciliary nerve (Plate **1**, Fig. 1. *n. cil. oc'mot.*) is given off. This runs parallel with the optic nerve, and penetrates the sclerotic coat of the eyeball. On its way, the ciliary nerve gives rise to a variable number of branches of microscopical size (Plate **1**, Fig 2, *rm. n. cil. oc'mot.*), which accompany it to the eye. The ciliary nerve receives, about one mm. distal to the region of the cells of the ciliary ganglion, a slender communicating ramus (Figs. 1 and 2, *rm. comn.*) from the ophthalmic branch of the fifth nerve.

*Trochlear Nerve.* The nidulus of the trochlear nerve is found in the somatic motor column in the ventral part of the mesencephalon, posterior to the nidulus of the oculomotor. The nerve (Fig. 1, *n. troch.*) takes its superficial origin from the dorsal surface of the brain, between

the optic lobes and the base of the epencephalon. Turning ventrad and cephalad, it passes through the orbit, running mediad and dorsad of the posterior and dorsal rectus muscles, and ends on the ocular face of the dorsal oblique muscle (Fig 1, *mu. ob. d.*).

*Abducent Nerve.* Like the other eye-muscle nerves, the abducens has its nidulus in the somatic motor column. It lies in the ventral part of the metencephalon, and from it the abducent neuraxons run ventrad to emerge from the ventral face of this division of the brain, not far from the median plane. The trunk of the nerve (Fig. 1, *n. abd.*) proceeds cephalad, and, crossing dorsad of the oculomotor nerve, divides into several terminal ramifications, which are distributed to the portion of the posterior rectus muscle lying laterad of the ophthalmic branch of the trigeminus (*rm. opth. trig.*), which passes through the proximal part of the muscle. Shortly before terminating in this way, the abducens sends cephalad a branch (Plate 1, Fig. 2, *rm. mu. qd.* + *pyr.*) to the muscles of the nictitating membrane. This branch soon bifurcates, and between its forks the communicating ramus connecting the trigeminal and oculomotor nerves often passes.

*Ophthalmic Branch of the Trigeminal Nerve.* The Gasserian ganglion presents two well-marked divisions, an ophthalmic portion (Fig. 1, *gn. Gas.*) extending cephalad, and a maxillo-mandibular portion directed ventrad. The first becomes gradually narrowed into the ophthalmic branch of the trigeminal nerve (*rm. opth. trig.*) which, running cephalad, usually penetrates the posterior rectus muscle, and then passes, ventrad of the proximal ends of the dorsal rectus and dorsal oblique muscles, to the anterior boundary of the orbit. Here it divides, one large nasal ramus (*rm. na.*) entering the nasal chamber, while several small branches (*rm. f.*) extend dorsally to a more superficial distribution, and, taken together, correspond to a frontal ramus.

From a point opposite the distal extremity of the ciliary ganglion, the ophthalmic branch sends out a communicating ramus (Figs. 1 and 2, *rm. comn.*), which unites with the oculomotor ciliary nerve about one mm. distad of the ciliary ganglion. However, not all the neuraxons which here leave the ophthalmic branch reach the oculomotor ciliary nerve, since a slender bundle of them emerges from the communicating ramus about midway in its course, and proceeds toward the eyeball as an independent trigeminal ciliary nerve (Fig. 2, *n. cil. trig.'*). In some cases, another trigeminal ciliary nerve (*n. cil. trig.''*) leaves the communicating ramus near its union with the oculomotor ciliary nerve, and likewise passes as a separate fibre to the eyeball.

### c. Histology.

#### 1. *Oculomotor Nerve.*

The trunk of the oculomotor nerve is made up of both large and small medullated neuraxons. Some of the former may reach the size of 15 micra in diameter, while some of the latter may measure only 3 micra. Between these two extremes all intermediate sizes are to be found. The trunk of the nerve is composed mainly of comparatively large neuraxons, among which a few small ones are interspersed, but at its lateral periphery a zone of small neuraxons occurs (Plate 7, Fig. 21). This zone is represented by the shaded portions of the diagrams shown in Plate 2, Figure 3, which represent cross-sections of the oculomotor at various levels along its course. The unshaded portion of the nerve trunk is that in which large neuraxons predominate. At *A* is shown the conditions which obtain in the nerve proximal to its branches. Diagrams *B* and *C* represent, respectively, sections through the origin of the branch to the dorsal rectus, and through the origin of the ventral ramus. It will be noticed that both draw their neuraxons from the unshaded portion of the nerve trunk. A photomicrograph of a cross-section of the branch to the dorsal rectus muscle is given in Plate 7, Figure 22. While the branch is mainly made up of neuraxons of large size, a certain number of smaller ones is also present. It is probable that the differences in size of the neuraxons correspond with the differences in the degree of development of the muscle fibres to which they are distributed. It has been pointed out by C. J. Herrick ('99) that large neuraxons of the eye-muscle nerves of Menidia are connected with large muscle fibres, and those of lesser calibre with small muscle fibres. In the eye muscles of the hen, fibres of varying sizes also occur. In a later paper (C. J. Herrick, :02), the writer just cited has advanced the opinion that differences in the calibre and medullation of neuraxons frequently signify nothing more than a correlation with the degree of functional development of the peripheral end-organs.

The neuraxons which pass into the ciliary ganglion are those which form the peripheral zone, shown by the shading in the diagrams of Plate 2, Figure 3. They are of small calibre. Distal to the ganglion the ciliary nerve is likewise entirely made up of small neuraxons (*D*), the medullary sheaths of which are, however, well developed.

#### 2. *Abducent Nerve.*

The elements of the abducent nerve, when seen in cross-section, appear, for the most part, as large medullated neuraxons. As in the oculomotor

nerve, a few small neuraxons occur among the larger ones. The nerve trunk closely resembles in appearance that of the oculomotor, except that a peripheral zone of small neuraxons is not present.

The branch given off to the muscles of the nictitating membrane is composed almost entirely of neuraxons of large size.

### 3. *Ciliary Ganglion.*

The cells obtained from the ciliary ganglion by maceration and isolation answer to the description of the ciliary cells of the hen already given by Retzius ('81). They are large bipolar cells, the processes of which become heavily medullated a short distance from the cell body (Plate 2, Fig. 4). In a few instances the two neuraxons were seen to arise by a common stem, so that the ganglion cell may be said to be unipolar in character.

With the object of comparing the finer structure of the ciliary ganglion with that of cerebro-spinal and sympathetic ganglia, attempts were made to obtain an *intra-vitam* methylen-blue stain. Three trials were made, but in only one of these were the cells of the ciliary ganglion affected. The cells in this instance were not, however, deeply stained, and no pericellular baskets of fibrils were differentiated about them, such as have been demonstrated about the sympathetic cells of birds by Huber ('99) after injection of methylen-blue into the blood system.

Such evidence as I have been able to obtain as to the sympathetic or cerebro-spinal nature of the ciliary ganglion has resulted from the use of the vom Rath mixture. After fixation in this reagent, sections of spinal ganglia can with ease and certainty be distinguished from sections of sympathetic ganglia. Spinal cells average larger, some measuring as much as 60 micra in diameter. Cells of this size are never found in sympathetic ganglia. Large medullated neuraxons are given off by spinal cells, and portions of these occur in every section through the ganglia. The peripheral neuraxons of the sympathetic cells, though also medullated, are never of as large calibre as those of the spinal cells. But the most convincing and characteristic peculiarity of the sympathetic ganglia is the mass of fine fibrils which occurs in them, filling in the interstices between the cells, and obscuring, to some extent, their boundaries and cytoplasm. Though their relations to the cells are not well brought out by the vom Rath stain, these fibrils undoubtedly form the pericellular baskets which are known to be present in sympathetic ganglia. Owing to the absence of such an abundance of fibrous elements the spinal gan-

glia present a quite different appearance. The cells of these are unobscured, and their boundaries are sharply defined.

When the ciliary ganglion of the hen is prepared according to the vom Rath process, and sectioned longitudinally, it is seen, under the microscope, to be divisible into two regions (compare *A* and *B*, text Figure). Approximately two-thirds of the ganglion (*B*) is composed mainly of large, well-defined cells, around which very few pericellular fibrils occur. Some of these cells reach the dimensions of the largest of the spinal-ganglion cells. In this portion of the ganglion are found small, but heavily medullated, neuraxons. Of these, a part are evidently continuous with the neuraxons entering the ganglion from the oculomotor nerve. Others are plainly seen to leave the ciliary ganglion by the ciliary nerve, the greater part of which arises from this portion of the ganglion, and is made up of small, well-medullated neuraxons.

In the dorsal region of the ciliary ganglion, on the side toward the ophthalmic branch of the fifth nerve, occurs an accumulation (*A*) of small cells, which makes up approximately one-third of the entire volume of the ganglion. Here are found fine neuraxons, showing little evidence of medullation, and a quantity of delicate fibrils resembling those of the pericellular baskets of sympathetic ganglia, although not present in such profusion as in the sympathetic ganglia. A communicating ramus between the ophthalmic branch of the trigeminus and the oculomotor ciliary nerve has been mentioned. Longitudinal sections of the ciliary nerves show that the slender, very slightly medullated neuraxons which compose the communicating ramus divide into two sets upon reaching the ciliary nerve. One of these bundles turns toward the eyeball, and accompanies the ciliary nerve to its peripheral distribution. The other bundle is recurrent, being deflected toward the ciliary ganglion. Its neuraxons run parallel with those of the ciliary nerve, and enter that portion of the ganglion which is characterized by small cells and pericellular fibrils. From this region there is given off to the eyeball a bundle ($\alpha$) of fine neuraxons with but slight traces of medullation. These accompany the medullated neuraxons from the remaining two-thirds of the ganglion ($\beta$) as component elements of the ciliary nerve. In certain cases they may be found occurring in the form of a distinct, non-medullated bundle, running close beside the larger group of medullated neuraxons, but separated from the latter by perineurium.

The relations of the two parts of the ciliary ganglion are shown in a diagrammatic way in the accompanying figure.

In any attempt to assign the ciliary ganglion, on histological grounds,

to either the cerebro-spinal or sympathetic systems, it is plain that the two regions described above must be separately considered. In respect to the first or ventral region (*B*), it can be said that the size of its cells, the heavy medullation of both its central and peripheral neuraxons, and the comparatively small volume of the pericellular fibrils, agree with the conditions found in cerebro-spinal ganglia. The central and peripheral neuraxons are, however, of smaller calibre. It is probable that the large bipolar ganglion cells obtained by maceration methods,

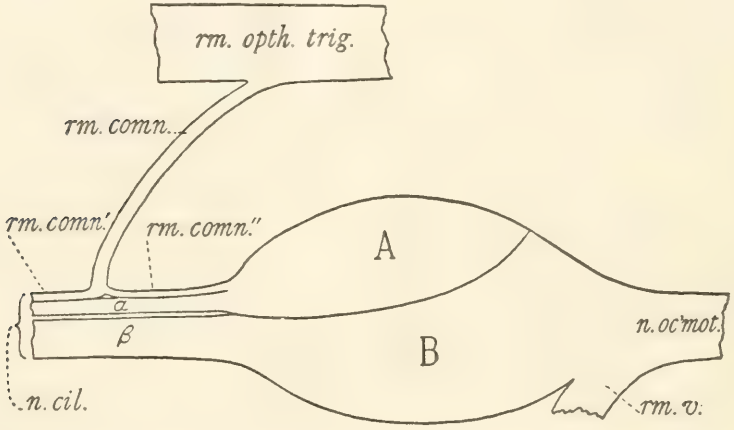


Diagram of a longitudinal section through the ciliary ganglion. *A*, region of small cells, non-medullated neuraxons, and pericellular fibrils; *α*, small, non-medullated ciliary neuraxons; *B*, region of large cells and small medullated neuraxons; *β*, small, medullated ciliary neuraxons; ***n. cil.***, ciliary nerve; ***n. oc'mot.***, oculomotor nerve (large and small medullated neuraxons); *rm. comn.*, communicating ramus (small non-medullated neuraxons); *rm. comn.'*, distal neuraxons of communicating ramus; *rm. comn.''*, recurrent neuraxons of communicating ramus; *rm. opth. trig.*, ophthalmic branch of trigeminal nerve; ***rm. v.***, ventral ramus (large and small medullated neuraxons).

as already described, were from this region. These resemble cerebro-spinal elements, except that two medullated processes instead of one are given off by each cell; and even where a tendency toward unipolarity occurs, the typical T-shaped condition of a cerebro-spinal neuron is not attained.

In the second or dorsal region (*A*) of the ciliary ganglion, the smallness of the cells, the absence of heavily medullated processes, and the comparative abundance of pericellular fibrils, suggest a sympathetic ganglion. Moreover, the resemblance is strengthened by the entrance

into this region of fine, very slightly medullated neuraxons from a gangliated dorsal nerve, the ophthalmic branch of the trigeminus. These neuraxons are identical in appearance with those of a typical ramus communicans passing, in the thoracic region of the fowl, to a sympathetic ganglion. A lack of correspondence between this portion of the ciliary ganglion and a typical sympathetic ganglion is to be recognized, however, in the comparative absence of medullation around the fine ciliary neuraxons given off distally. The post-ganglionic neuraxons of a sympathetic ganglion are also of small calibre, but in the hen they are well medullated. This lack of correspondence, due merely to differences in the degree of medullation, seems comparatively unimportant. It should be remembered, too, that in other classes of vertebrates non-medullated neuraxons are characteristic of post-ganglionic sympathetic nerves.

## PART II. — DEVELOPMENT.

### A. Historical Survey.

In reviewing the literature which deals with the development of the ciliary ganglion, it must be kept in mind that the name "ciliary" has been applied by various authors to *two* entirely distinct ganglia. One of these is found at the base of the first or ophthalmic branch of the trigeminal nerve, while the other is connected with the oculomotor nerve, and is the true ciliary ganglion of the adult.

The first of these ganglia arises in the following manner. In early stages of development, at least in sharks and birds, the most anterior portion of the neural crest becomes differentiated, in the region of the eye muscles, into an enlargement resembling the fundament of a cerebrospinal ganglion. In sharks this has been observed to fuse later with the anterior part of the Gasserian ganglion, and the same fusion doubtless takes place in birds. From the ganglion originating thus, the first branch of the trigeminus proceeds forward (van Wijhe, '82; Beard, '87; Neal, '98). For this ganglion of the ophthalmicus profundus in sharks Beard, in 1887, proposed the name "mesocephalic." Following Dohrn and Neal, I have adopted this designation, since all names previously applied to the ganglion in question have been used as synonyms for the ciliary. Indeed, as already stated, the name ciliary itself has often been applied to it.

The separate development of the ganglion of the ophthalmic branch of the trigeminus, and its subsequent fusion with the rest of the

Gasserian ganglion, have been observed in mammals and amphibians, as well as in fishes. Chiarugi ('94, '97) makes such an assertion for the embryos of guinea-pigs, and Ewart ('90), mentions the discovery, in a five-months' human embryo, of vestiges of an "ophthalmicus profundus ganglion" lying under cover of the inner portion of the Gasserian. Brauer (:04) describes the development of the "ganglion ophthalmicum" and of the "ganglion maxillo-mandibulare" in the Gymnophiona as independent of each other. Hoffmann ('85) considers the Gasserian ganglion in both embryonic and adult reptiles as divisible into two parts. In some of the lower vertebrates, as cyclostomes (Dohrn, '88; von Kupffer, '95) and ganoids (Allis, '97), these two ganglia, the mesocephalic and the maxillo-mandibular, retain complete independence throughout life.

There is good evidence, then, that a distinct ganglion, the mesocephalic, is developed throughout the vertebrate series in connection with the ophthalmic division of the fifth nerve. This ganglion, except in the cases of certain low forms, soon becomes fused with the common ganglion of the maxillary and mandibular divisions to form the Gasserian ganglion of the adult.

The true ciliary ganglion appears much later than the mesocephalic, and is always more or less directly connected with the third cranial nerve. The various ways in which its development has been said to take place will be outlined in the following pages. It will be sufficient, at present, to say that all writers whose statements are based on actual observation agree that it does not arise, like the mesocephalic and other cerebro-spinal ganglia, through direct differentiation from the cells of the neural crest.

In the reviews which follow I shall summarize in chronological order the observations already made on the development of the oculomotor and abducent nerves and the ciliary ganglion in the five classes of vertebrates. As far as possible, I shall distinguish between the mesocephalic and the ciliary ganglia. Each author's nomenclature will first be given, and then, if the identities of the ganglia are clear from his description, the terminology adopted above will be substituted for the sake of clearness and uniformity.

### 1. Fishes.

The first investigations upon the development of the oculomotor and abducent nerves in fishes were made by Marshall in 1881. In the embryo shark (Scyllium canicula) Marshall ('81) found that, in Balfour's

stage *K*, the third nerve arises from the ventral face of the mid-brain by a root triangular in shape, containing many "nerve cells." Running caudad and laterad, the nerve reaches the interval between the dorsal ends of the first and second head cavities, where it expands into a small ganglion. At this ganglion, the nerve divides into two main branches, one running cephalad along the top of the first head cavity to the extreme anterior end of the head, the other passing ventrad between the first and second cavities. A short branch, coming directly from the Gasserian ganglion, enters this small ganglion and later, uniting with the first branch of the oculomotor described above, forms the ramus ophthalmicus profundus of the adult; this ramus has, in most cases, the appearance of being a branch of the trigeminal nerve. From the posterior wall of the first head cavity are derived those muscles of the eyeball which later are innervated by the oculomotor. The author considers this small ganglion between the tops of the first two head cavities to be the ciliary ganglion of the adult, and agrees with Schwalbe that it belongs exclusively to the third nerve.

It is evident from later investigations by others that Marshall is here dealing with the mesocephalic ganglion, and that his first branch of the oculomotor has only an apparent connection with that nerve, being, as a matter of fact, the ramus ophthalmicus profundus of the trigeminus, with which the mesocephalic ganglion is primitively connected. The close contact into which the two nerves come in the region of the mesocephalic ganglion accounts for the author's failure to separate them. (Comp. van Wijhe, '82.)

The sixth or abducent nerve was first observed in stage *O*, some time after the oculomotor had made its appearance. It springs from the ventral face of the hind-brain by a large number of slender roots, and runs to the fundament of the posterior rectus muscle. The roots as well as the trunk of the nerve contain many more or less elongated, fusiform cells, but none of these are ganglion cells.

Marshall and Spencer ('81) confirm Marshall ('81) without adding anything of importance to the latter's account.

In 1882 van Wijhe published his excellent description of the development of cranial nerves in selachians. In Balfour's stage *I* he found the fundament of a ganglion connected with the ophthalmic branch of the trigeminus, and representing the anterior extremity of the neural crest. This ganglion — called the ciliary by van Wijhe, but evidently the mesocephalic of our nomenclature — at first lies immediately under the epidermis, but soon moves away from it in the direction of the gan-

glion of the second and third branches of the trigeminus. Not until the next stage *J*, does the oculomotor appear; it then arises by a broad proximal end from the mid-brain. Passing ventrad the third nerve crosses the ophthalmic branch of the fifth on its median side at the level of the mesocephalic ganglion, to which it becomes closely applied; but, according to van Wijhe's view, only a close contact, not an actual union, occurs. Later, the oculomotor nerve and the mesocephalic ganglion draw away from each other, although a slender communicating nerve continues to connect the two.

But van Wijhe's most important contribution to the subject was the discovery of a second ganglion, which, in stage *O*, appears as a dumb-bell-shaped mass of cells placed on the branch of the third nerve which supplies the ventral oblique muscle, in the position occupied by the ganglion oculomotorii described by Schwalbe ('79) in adult selachians. This ganglion oculomotorii (which is our ciliary) is comparatively remote from the mesocephalic ganglion, and the author emphasizes the lack of connection between the two. On account of its late appearance, and the presence of a small branch from it to the arteria ophthalmica, he considers the ganglion as belonging to the sympathetic system, but gives no account of its actual development. The mesocephalic ganglion he regards as homologous with a spinal ganglion. The origin of the oculomotor from the base of the brain, the time of its appearance, its histological structure, its lack of a true ganglion in early stages, and its crossing, if not union, with a dorsal root distal to the ganglion of the latter, seem to the author to prove that the nerve in question is a purely ventral one.

To Marshall's account of the comparatively simple development of the sixth nerve van Wijhe added practically nothing.

Beard ('85) describes the development in elasmobranchs of what he then called the ciliary ganglion, but later termed the mesocephalic. This account was repeated and supplemented in his notable paper of 1887, to which reference has been made. In this paper he clears up the existing confusion caused by the various names given to the ganglia developing in connection with the oculomotor nerve and the ophthalmic branch of the trigeminus. The first he shows to be the true ciliary ganglion of the adult, and, therefore, entitled to that name; and for the second he proposes, as already stated, the name mesocephalic. He describes the way in which, in elasmobranchs, the mesocephalic ganglion, deriving its cells partly from the neural crest and partly from the ectoderm in the region of a primitive branchial sense organ, gradually recedes from the skin, and fuses with the maxillo-mandibular ganglion.

As this change of position takes place, a nerve — the ophthalmicus profundus — is developed, connecting the mesocephalic ganglion with the branchial sense organ. The oculomotor appears later than the ophthalmicus profundus, and never has any direct connection with the mesocephalic ganglion, although, during development, it is for a time closely applied to the latter. The true ciliary ganglion is not present until much later. When it does appear, the mesocephalic ganglion and the oculomotor nerve are connected by a small communicating branch, probably corresponding to the radix longa of higher animals; and it is near the entrance of this branch into the third nerve that the ciliary ganglion is first to be seen. Beard did not follow the development of the ganglion step by step, but calls attention to the assertion of Hoffmann ('85), that it arises in reptiles as an outgrowth of the ophthalmic (mesocephalic) ganglion. He favors the view that it belongs to the sympathetic system.

Phisalix ('88, '88[a]), mistaking the mesocephalic for the ciliary ganglion in skate embryos, asserts that at first the oculomotor nerve and its ganglion are independent of each other. The ganglion is said to result from the dividing into two of the ganglion of the trigeminal nerve before the oculomotor has appeared.

Ewart ('90) gives us the first account of actual observations on the development of the ciliary ganglion in fishes. He finds that, at a certain stage in skate embryos, a slender outgrowth from the inferior border of the ophthalmicus profundus (mesocephalic) ganglion meets and blends with the descending (ventral) branch of the oculomotor. This outgrowth is crowded with cells, while the fibres of the descending branch of the oculomotor, as well as its root and trunk, are "absolutely destitute of cells." Later, cells accumulate at the junction between the outgrowth and the oculomotor, as if the intermingling of the two sets of fibres formed a network which resisted the further migration of cells from the mesocephalic ganglion. At a still later stage, in typical cases, all the ganglion cells are seen to have left the outgrowth, and to have accumulated on the oculomotor as a rounded mass, from which ciliary nerves take their origin. The ganglion thus arising, plainly the ciliary, stands, therefore, in the relation of a sympathetic ganglion to a dorsal cranial nerve, the ophthalmicus profundus.

Dohrn ('91) takes up the matter of the histogenesis of the oculomotor more fully than previous writers on the eye-muscle nerves, and also offers an entirely new explanation of the origin of the ciliary ganglion. He observed (p. 3) that in the embryos of selachians the third nerve

first makes its appearance as a number of crowded pale cells in the marginal veil of the mid-brain. The plasma of these cells emerges from the ventral side of the neural tube as fine processes, which unite to form an irregular network. The meshes of this network are extended by the fusion of the processes of large cells, the nuclei of which lie in the plasma mass. The network stretches out through the mesenchyme, and gives rise, in the vicinity of the front end of the chorda dorsalis, to the small trunk of the oculomotor nerve. Dohrn's view of the structure of the growing nerve is in general accordance with the opinions of Balfour, Marshall, Beard, von Kupffer, and others, who support the "chain theory" of nerve formation. To prove that the medullary tube is the source of these cells whose processes make up the nerve trunk, Dohrn devotes much space and many figures. He shows that cells may be observed in the root of the oculomotor nerve, half in and half out of the medullary tube. Although he is obliged to admit that with present staining methods it is not possible, in early embryonic stages, to distinguish emigrating medullary cells from the surrounding mesodermal cells, he calls attention to the fact that the nuclei of the nervous network are larger than the nuclei of nearly all the neighboring mesodermal cells. Numbers of rounded and oval nuclei are to be seen in the course of the oculomotor before this grows down and connects with the mesocephalic ganglion, the long axes of these nuclei being perpendicular to those of the nuclei of the ganglion. The nuclei lying along the third nerve cannot, therefore, be considered derivatives of the mesocephalic ganglion.

In older embryos there occur, in the course of the third nerve, groups of differentiating ganglion cells, corresponding in position to the ganglia found along the oculomotor in the fully grown animal; and the development of these cells can be followed with certainty until the adult condition is reached. The ganglion cells arising in this way Dohrn believes to have been originally migrant medullary cells.

After considering the definitions which have been given to cerebro-spinal and sympathetic ganglia, Dohrn reaches the conclusion that in the diffuse ciliary ganglion of selachians we have a ganglion which, because of its unique origin from emigrant medullary cells, belongs neither to the cerebro-spinal nor to the sympathetic systems.

In its histogenesis, the abducent nerve was found to resemble closely the oculomotor. Cells were discovered, wandering out into its roots from the ventral wall of the hind-brain, but in the case of this nerve none of these become ganglion cells in the adult.

Following the article of Dohrn just cited, three investigators published in rapid succession accounts of the development of the oculomotor nerve in selachians. These accounts were remarkable for the fact that they agreed in ascribing to the third nerve an extraordinary origin, the possibility of which had apparently never occurred to other investigators.

Platt ('91) describes, in embryos of Acanthias vulgaris, a line of nerve cells extending cephalad from the trigeminal ganglion, and soon enlarging into the fundament of a ganglion, which she terms the ciliary. This ganglionic fundament, first meeting an anterior prolongation from the neural crest (which develops into the transitory "thalamic nerve"), finally ends in a mass of cells connected with the primary nasal epithelium. This line of cells is later represented by the ramus ophthalmicus profundus trigemini. (From the foregoing description I consider it probable that Platt's "ciliary" ganglion is the same as Beard's mesocephalic.) From the inner (median) cells of this ganglion, the oculomotor nerve takes its origin as a cellular proliferation, which grows from the ganglion toward the brain, with which it becomes united in the floor of the mid-brain. Two figures are given showing the oculomotor when it consists of but a single cell. The author concludes that the third nerve is, therefore, primarily sensory, the mesocephalic ganglion being at this time connected with a patch of thickened epithelium, and no muscle cells having as yet appeared in the walls of the premandibular cavity.

Mitrophanow ('93) followed with a confirmation of Platt's account of the origin of the oculomotor. He observed this peculiar development of the nerve in embryos of Raja, Torpedo, and Pristiurus. The ganglion from which the third nerve grows, as a cordon of cells, to the brain is plainly the mesocephalic, but the author prefers to call it the ciliary, although he indicates his familiarity with the name mesocephalic by mentioning it several times as a synonym for ciliary.

Finally, Sedgwick ('94) maintains that nerves do not develop as processes from central cells, according to the view of His, but arise through the differentiation of a reticular substance already in position. The oculomotor is formed in elasmobranchs as a differentiation of this reticulum, resulting from the breaking up of the neural crest, and first appears as a forward projection of nuclei from the ciliary (mesocephalic) ganglion. In the author's words (p. 96), "The third nerve, therefore, presents this interesting and remarkable peculiarity in Scyllium and Acanthias; it grows or is differentiated from the ciliary ganglion to the floor of the mid-brain and not in the opposite direction, as has hitherto been supposed." Sedgwick publishes no figures in support of his contention.

In his study of the development of the cranial nerves of Ammocoetes, von Kupffer ('91) assigned to the nerve he believed homologous with the oculomotor of higher vertebrates both dorsal and ventral roots and spinal as well as sympathetic ganglia. In a later paper (von Kupffer, '95) he states that the oculomotor in Ammocoetes appears to be a ventral nerve with which is related dorsally the anterior half of the first trigeminal ganglion.

The third nerve and the ciliary ganglion in embryos of Amia calva are described by Allis ('97). This writer has, however, no positive information to offer as to the early stages of their development.

Hoffmann ('97) states that the oculomotor, when first detected in Acanthias vulgaris, appears as a fibrous ventral root. At this time there is no ganglion at the proximal end of the nerve. His observations on the abducens confirm those of Marshall and van Wijhe.

Chiarugi ('97) declares, in opposition to Mitrophanow, that the third nerve in selachians grows centrifugally from the base of the mid-brain.

By the use of his modification of the vom Rath method, Neal ('98) demonstrated in so convincing a manner the neuroblasts of the oculomotor and their processes in Squalus acanthias that he removed all doubt as to the origin of the nerve from the ventral wall of the mid-brain. The neuroblasts showed the characteristics of those described by His in the spinal cord, and their darkly staining processes could be followed partly into the mesenchyme, where they were grouped to form the nerve trunk, and partly in a posterior direction within and parallel to the medullary wall, where they took part in the formation of the ventral fibre tract. The nerve growing out in this fashion from the mid-brain exhibits many nuclei lying peripherally along its fibres. It soon connects with the cells of the mesocephalic ganglion. Whereas the nerve is several cells in thickness near the ganglion, its calibre grows less toward the brain wall, a condition which, if one were unacquainted with its earlier history, might lead to the supposition that the growth of the nerve takes place from the ganglion toward the brain. Cells were observed migrating out from the mesocephalic ganglion and adhering closely to the oculomotor fibres. The fate of these cells was not determined, nor was the development of the ciliary ganglion followed. No entirely satisfactory evidence of migration of medullary elements was observed.

The abducent nerve was found by Neal to arise in the form of a slender bundle of neuraxons from neuroblasts situated in the ventral horn of the medulla. The number of its roots increases during devel-

opment from one to three or four. The nuclei seen along its course are distinctly peripheral in relation to its fibres. There were no convincing indications of the migration of cells from the neural tube.

The development of the third nerve in selachians was described by Hoffmann ('99), but he added nothing new to the subject. The nerve in question grows down from the mid-brain and anastomoses with the ganglion ophthalmicus. This is the mesocephalic ganglion of Beard ('87), with whose article the author in the main agrees; but he considers the name ophthalmicus preferable to mesocephalic. Later, the oculomotor nerve and the mesocephalic ganglion draw apart, remaining connected, however, by a ramus anastomoticus. Presently, two ganglia appear on the third nerve, corresponding in position to those described by Schwalbe ('79) for adult selachians. Although the author was not able to work out the development of the ganglionic groups appearing on the oculomotor, he believes the account of Ewart ('90) to be correct, and considers the ciliary the most anterior sympathetic ganglion of the head.

Allis (:01, p. 131) mentions the presence of small and large cells in the ciliary ganglion of an embryo of Mustelus lævis. The large cells resemble cerebro-spinal ganglion cells, and the author suggests the probability that both spinal and sympathetic elements enter into the composition of the ciliary ganglion. Long and short roots were distinguished, but no extra-cranial sympathetic root could be made out.

### 2. Amphibians.

Almost nothing is known of the development of the third and sixth cranial nerves and the ciliary ganglion in amphibians. Johnson and Sheldon ('86, p. 94) state that in the embryo newt the oculomotor arises, like certain other of the cranial nerves, as an outgrowth of the neural crest, but no details of the process are given. According to Marshall ('93, p. 133) the oculomotor is present as a slender nerve in tadpoles of the frog at the time of the opening of the mouth. It arises from the lower part of the side of the mid-brain, not far from the median plane, and has already the course and relations of the nerve in the adult. Its early development has not been ascertained.

### 3. Reptiles.

Our knowledge of the development of the eye-muscle nerves and the ciliary ganglion in reptiles is derived almost entirely from the extended accounts of the two investigators, Béraneck and Hoffmann, both of whom studied embryos of Lacerta agilis.

Béraneck ('84) found the oculomotor, in an early stage of development, arising from the ventral face of the mid-brain by a triangular root crowded with cells possessing round nuclei, "et par touts leurs caractères se rapprochent beaucoup des cellules médullaires." Whether this cellular accumulation at the root of the nerve is really ganglionic, or whether its cells, like those distributed along the nerve, later form the sheaths of the fibres, could not be determined. At the proximal termination of the oculomotor occurs a little cellular mass, which the author believes to represent the ciliary ganglion. At this stage no communicating branch exists between this or any other part of the third nerve and the ophthalmic branch of the fifth.

In later stages, numerous cells are to be seen distributed along the whole nerve, those at the broad root being rounded and closely resembling the medullary cells, while those more distally situated are fusiform, with their long axes parallel to the nerve fibres. These differences in shape among the cells of the oculomotor are more apparent the older the embryo. The cells are more abundant in the proximal than in the distal part of the nerve trunk. The approximately spherical ciliary ganglion incloses round cells with distinct nuclei and fine granules. It is now connected by a slender ramus with the ophthalmic division of the trigeminal nerve. From the anterior face of the ciliary ganglion there runs cephalad, for an undetermined distance, a fine bundle of fibres, which the author believes to represent the ophthalmic branch of the oculomotor described in sharks by Marshall ('81). In old embryos, the ciliary ganglion shows the relations to the third nerve found in the adult: it is situated a little on one side of the nerve trunk, to which it is attached by a very short and thick bundle of nerve fibres.

Nowhere in his account does Béraneck advance the theory that the cells along the third nerve may have been derived through migration from the neural tube, nor does he express an opinion as to the source of the cells which differentiate *in situ*, in the oculomotor, into the cells of the ciliary ganglion.

The abducens appears somewhat later than the oculomotor, springing as a slender nerve from the ventral face of the hind-brain. During its development it presents but a single root, fibrillar in character, and destitute of cells, except for a few mesodermal elements which surround it externally. The same conditions are found along the course of the nerve trunk, the only cells connected with it being of mesodermal origin, arranged in a single layer about its periphery.

The account of the development of the ciliary ganglion given by Hoffmann ('85) is at variance with that of Béraneck, although both authors used as material the same species of lizard, namely, Lacerta agilis. While Hoffmann saw the younger stages of the development of the ganglion in snake embryos, the entire process was worked out in lizards only. According to his observations, the anterior part of the neural crest gives rise to two ganglia, that of the first branch of the trigeminus — the ophthalmic (mesocephalic) — and the ganglion common to the second and third branches — the Gasserian. The oculomotor develops later than the trigeminus, springing by a broad base from the ventral surface of the mid-brain. It is composed of a small amount of finely striated protoplasm, containing many nuclei closely crowded together. Passing on the median side of the mesocephalic ganglion, the nerve sends out to the anterior face of the latter a communicating ramus. By the aid of several drawings and a series of diagrams, the author shows that a large mass of cells is proliferated from the distal end of the mesocephalic ganglion, that this mass separates from the parent ganglion, and, guided by the communicating ramus, makes its way to a point close to the third nerve, with which it becomes united by a very short and thick bundle of fibres. This mass of cells becomes the ciliary ganglion of the adult, and the bundle of fibres binding it to the third nerve, the radix brevis. The ganglion retains connection with the fifth nerve through a slender radix longa, which, however, does not end in the mesocephalic ganglion, but in the ramus nasalis, a branch of the ophthalmic nerve, which grows out from the distal extremity of the mesocephalic ganglion while the ciliary ganglion is undergoing development. Hoffmann is convinced of the sympathetic nature of the ciliary ganglion, basing his opinion on the late appearance of the ganglion, its origin from the homologue of a spinal ganglion, and its development through the participation of both sensory and motor nerves, one, the ophthalmic, arising by a true dorsal root, the other, the oculomotor, by a true ventral root.

C. L. Herrick ('93) gives a figure of the developing oculomotor nerve in a snake embryo, showing migration of nuclei from the mid-brain into the root of the nerve. These nuclei he holds to be those of cells which, outside the neural tube, produce the nerve fibres.

### 4. Birds.

Remak ('51) and His ('68, '79) describe and figure in chick embryos, between the third and fifth days, a cellular prolongation extending

cephalad from the Gasserian ganglion, and swelling into a crescentic enlargement near the eye vesicle. This structure is regarded by His as the persistent neural crest in the anterior head region. Both authors, without tracing its fate, assume that the crescentic terminal enlargement is the ciliary ganglion. Kölliker ('79) expresses himself as also of this opinion, although he acknowledges the insufficiency of the evidence on which the assumption is based. That the ganglion in question is the mesocephalic, and not the ciliary of the adult, is beyond doubt.

The first study of the development of the eye-muscle nerves of vertebrates was made by Marshall ('77, '78) on chick embryos. He found that the neural crest forms, at the twenty-ninth hour of incubation, a prominent outgrowth above the mid-brain. At forty-three hours this outgrowth is directed ventrad and lies in close contact with the walls of the mid-brain; and at fifty-three hours a large mass of cells is to be found connected with the mid-brain, and about half-way down its side. In a sixty-hours' chick, the oculomotor nerve arises from the ventral surface of the mid-brain, but is farther from the median plane than at later stages. From this evidence the author is "led to the belief that the third nerve is developed directly out of the outgrowth from the top of the mid-brain" seen at the twenty-ninth hour, and "that, at some period between the forty-third and sixtieth hours, its attachment shifts down from the top of the mid-brain to the lower part of its sides" (p. 25). Rabl ('89) accepts, on theoretical grounds, this view of the origin of the third nerve.

Longitudinal sections of a ninety-six-hours' chick show the oculomotor as a large nerve, arising from the ventral face of the mid-brain. Its base is "ganglionic," and it terminates a little posterior to the optic nerve, in a "ganglionic" swelling. From its enlarged distal end two branches are given off, one passing cephalad and dorsad to the fundament of the posterior rectus muscle, the other continuing the course of the main trunk, crossing the ophthalmic nerve nearly at right angles, and passing caudad and ventrad of the optic nerve. No mention is made of a connection between any part of the third nerve and the ophthalmic division of the fifth.

In a subsequent paper (Marshall, '81) the author accepts the view advanced by Schwalbe ('79) that this terminal swelling of the trunk of the oculomotor represents the ciliary ganglion of the adult. He advances no opinion as to the source of the cells of the ganglion.

Marshall's observations on the sixth nerve were incomplete. It was first detected in an embryo of ninety-three hours. Unlike the oculo-

motor, it arises by a series of slender roots instead of a single, large "ganglionic" root. It is a very slender, cellular nerve and does not branch.

Foster and Balfour ('83, p. 128) make brief mention of a connection between the ophthalmic branch of the trigeminus and the oculomotor in the chick, soon after the third day of incubation. They merely state that, near the eye, the ophthalmic branch of the fifth nerve "meets and unites with the third nerve, where the ciliary ganglion is developed."

The view of Remak and His in regard to the development of the ciliary ganglion from the neural crest is shared by Goldberg ('91). Like them, he mistakes the mesocephalic ganglion for the ciliary.

Goronowitsch ('93), on the other hand, declares that the primary neural crest completely disappears in bird embryos during an early period of development. The ciliary ganglion arises later, and quite independently of the neural crest. The writer did not observe the actual process by which it is developed, but is inclined to accept the explanation given by Dohrn of its origin in selachians. He states that he himself has observed in teleosts abundant evidence of the migration of cells from the medullary tube into the root of the third nerve.

D'Erchia ('95) gives a description and a figure of a communicating nerve between the ophthalmic branch of the trigeminus and the ciliary ganglion in an embryo chick of twelve days' incubation. He, therefore, denies Schwalbe's assertion that no sensory root of the ciliary ganglion exists in birds. This difference of opinion is easily explained by the fact that D'Erchia made his observations on embryonic, and Schwalbe on adult material. While present in the embryo, this *direct* connection between the trigeminal nerve and the ciliary ganglion does not persist in the adult. (See Plate 1, Figs. 1 and 2.)

Rex (:00) observed that in embryos of the duck the ciliary ganglion first makes its appearance as a distinct thickening in the course of the oculomotor nerve, due to the presence of an accumulation of ganglion cells. He did not follow the development of the ganglion.

### 5. Mammals.

The observations on the genesis of the eye-muscle nerves and the ciliary ganglion in mammals have been fragmentary.

In sections of rabbit embryos, Kölliker ('79) discovered the oculomotor arising at the earliest stage observed from the lateral, not the ventral, face of the brain. It showed no evidence of a ganglionic swell-

ing at its base, and no cells were intermingled with its fibres. About its periphery was a thin envelope, formed by a single layer of mesodermal cells. Later, the nerve was observed to have descended to the ventral face of the brain.

His ('80, '88, '88[a]) maintains that the ciliary ganglion develops in man from the anterior portion of the first ganglionic or trigeminal complex of the head, which is a direct descendant of the neural crest. He does not accept the distinction made by Beard ('87) between a mesocephalic and a ciliary ganglion, but asserts that the ganglion which Remak, himself, and so many others have seen at the anterior extremity of the neural crest, is identical with the long-known ciliary ganglion. In opposition to Schwalbe, he assigns this ganglion to the fifth rather than to the third nerve, since it develops over the fore-brain, while the third nerve grows out from the ventral face of the mid-brain. Furthermore, the oculomotor arises as a purely motor nerve, and, as such, is not entitled to a ganglion.

Both the third and sixth nerves arise in human embryos as fibrous outgrowths of neuroblasts situated in the ventral zone of the medullary wall, not far from the median plane.

It is stated in Quain's Anatomy ('95, Thane, p. 388) that Martin ('90) found a dorsal root of the oculomotor nerve in an embryo cat. The original article has not been accessible.

His, Jun. ('91) compared, in an embryo cat, the cells of a cerebrospinal, a sympathetic and the ciliary ganglion. In the first, he found large, bipolar cells, and in the sympathetic and ciliary ganglia, small, unipolar cells.

Chiarugi ('94, '97) observed the oculomotor nerve, in the youngest guinea-pig embryos in which it was present, springing from the ventral side of the mid-brain, near the median plane. On the trunk of the nerve, close to the root, he discovered a rudimentary ganglion. Since no ganglion cells were to be seen along the further course of the nerve, between the ganglion and the place of connection with the ophthalmic branch of the trigéminus, he considers it improbable that the ganglion owes its origin to nervous elements which have passed from the trigeminus to the oculomotor. In the wall of the brain there were to be seen, however, several neuroblasts, lying along the root fibres of the third nerve, and, apparently, advancing to the free surface. This leads him to the supposition that the cells of the ganglion are derived through migration from the brain wall. He believes that this ganglion has no connection with the ciliary ganglion, which develops later in close re-

lation with both the inferior branch of the third nerve and a communicating ramus passing to it from the ophthalmic branch of the fifth. He states that he found the origin of the ciliary ganglion difficult to trace, but is inclined to think that its cells come from the ophthalmic branch of the trigeminus. At the origin of the communicating branch from the latter nerve, a small cluster of ganglion cells was found.

Throughout the whole length of the third nerve, there could be seen, disseminated among the nerve fibres, nuclei of cells, the interpretation of which the writer found very difficult.

The ciliary ganglion was recognized by Dixon ('95) as a distinct cellular mass in a human embryo of the sixth week. It appears at first to be more closely connected with the frontal and fourth nerves than with the nasal and third nerves. It later shifts its position, and, by the eighth week, has established connections as in the adult. (Comp. Reuter, '97.)

Reuter ('97), though concerned chiefly with the development of the eye muscles in the pig, furnishes some interesting information in regard to the early stages of the ciliary ganglion. He discovered, in an embryo measuring 14 mm. from nape to rump, differentiating ganglion cells lying in the oculomotor nerve, both at the place where it divides into its terminal branches and in the course of its long branch to the ventral oblique muscle. In a later stage these cells are accumulated in one mass in the form of a distinct ciliary ganglion. At the time of the first appearance of the cells, no connection exists between the oculomotor and the first branch of the trigeminus. Later, a radix longa is developed. The writer obtained no clew as to the source of the cells of the ciliary ganglion, but, in view of the latter's late development, he considers it very unlikely that its cells have any genetic connection with the neural crest or the Gasserian ganglion. He is of the opinion that His ('88[a]) and Dixon ('95) have mistaken for the ciliary ganglion the fundament of the dorsal oblique muscle during the period between the disintegration of the neural crest and the first appearance of the ganglion.

It is evident from the foregoing reviews that, while observers agree in the main as to the development of the abducent nerve, there is a wide diversity of opinion in the cases of the oculomotor nerve and the ciliary ganglion. Consequently, as far as the latter structures are concerned, it is difficult to draw from the existing literature satisfactorily supported generalizations. Especially is this true of the ciliary ganglion,

which appears to vary in its manner of development not only among animals belonging to different classes of vertebrates, but also among animals belonging to the same class. Even in the same species the accounts of its origin, in one instance, disagree (comp. Béraneck, '84, and Hoffmann, '85). The weight of the evidence, however, seems to justify the following general statements: —

1. The oculomotor nerve develops after the manner of a ventral spinal nerve from the floor of the mid-brain. Its histogenesis has been described both in accordance with the "process theory," i. e., formation by neuraxons growing out from centrally situated neuroblasts (Neal); and in accordance with the "chain theory," i. e., formation by chains of cells which anastomose in the mesenchyme (Dohrn).

Marshall's theory of a primary connection between the third nerve and the neural crest is not supported by the facts, he himself being unable to trace satisfactorily the intermediate steps in the shifting of the nerve from a dorsal to a ventral position. Kölliker, it is true, finds the nerve in rabbit embryos at first half-way up the side of the neural tube, but his observation in this respect stands alone. Other investigators have so convincingly disproved the statements of Platt, Mitrophanow and Sedgwick, who hold that the third nerve grows from the mesocephalic ganglion toward the brain, that their erroneous conclusions must be set down to inadequate methods and mistaken interpretations.

2. The developing oculomotor nerve exhibits throughout its course numerous cells distributed among its fibres. Its proximal extremity is enlarged and crowded with cells.

With these statements all writers except Ewart and Kölliker agree. Ewart ('90) asserts that in skates at the time of the formation of the ciliary ganglion the oculomotor fibres are free from cells; and Kölliker ('79) finds no cells connected with the oculomotor in rabbit embryos, except a single peripheral layer which is of mesodermal origin.

3. The abducent nerve develops after the manner of a ventral spinal nerve, usually by several roots, from the ventral wall of the hind-brain. Numerous cells are associated with the nerve fibres in fishes and birds, but not in reptiles (Béraneck), and probably not in mammals.

The comparatively few observations on the genesis of the sixth nerve are all in general agreement.

4. The ciliary ganglion may originate either (*a*, sympathetic type of development) by the migration of ganglion cells from the mesocephalic ganglion into the oculomotor nerve, either directly or by way of the ophthalmic branch of the trigeminus (Ewart, Hoffmann, Chiarugi); or

(*b*, unclassifiable type of development) by the development of ganglion cells *in situ* in the third nerve (Dohrn, Béraneck, Rex, Reuter).

None of the investigators who have observed the formation of the ganglion *in situ* have advanced an opinion as to the source of its cells with the exception of Dohrn, who gives evidence of their derivation through migration from the wall of the neural tube.

5. In all vertebrates, at an early stage in the development of the of the ciliary ganglion, a connection, in the form of a communicating ramus, is established between it and the ophthalmic branch of the trigeminus.

## B. Observations.

### 1. Methods.

The two most satisfactory staining methods for the purposes of my study proved to be the mixture devised by vom Rath for fixing tissues and the Heidenhain iron haematoxylin stain. The vom Rath fluid used according to Neal's procedure (see Neal, :03) has the very desirable effect of differentiating neuroblasts and their growing processes. It colors but slightly other cells of the neural tube (ependymal cells, spongioblasts and indifferent cells), and the same is true of its effect on the cells of the mesenchyme. In preparing the fluid the formula used was that given in 1895 by vom Rath ('95, p. 283) :—

200 c.c. saturated solution of picric acid.

1 grm. platinic chloride, dissolved in 10 c.c. water.

2 c.c. glacial acetic acid.

25 c.c. 2 per cent osmic acid.

In this mixture, embryo chicks were allowed to remain for three days or more, during which time the fluid was once changed. They were then washed for a minute in two changes of methyl alcohol, and placed for from twenty-four to forty-eight hours in a 0.5 per cent solution of pyrogallic acid, which intensified the stain. From this reagent the embryos were brought up slowly through the different strengths of alcohol to absolute, then cleared in xylol, and embedded in paraffin. This treatment rendered the material very brittle, and careful handling was necessary in all operations subsequent to immersion in the fixing fluid. After serial sectioning and fixation to the slides, no treatment for the further staining of the tissues followed. The paraffin was dissolved in xylol, and the sections were immediately mounted in xylol-balsam. The preparations were allowed to dry uncovered, since the use of cover glasses

is likely to be followed by an alteration in the stain, resulting in a faded or yellowish appearance, and loss of good differentiation.

Heidenhain's iron haematoxylin stain was employed in the usual way after fixation either in Zenker's fluid, or in a saturated aqueous solution of corrosive sublimate to which had been added 1 per cent glacial acetic acid. Besides giving its well-known sharp nuclear stain, iron haematoxylin differentiates clearly the primitive fibrils as soon as these begin to appear in developing nerves. It is less favorable than vom Rath's mixture for the earliest stages of nerve formation, as it is not a selective stain for the processes of the neuroblasts. After fixation in Zenker's fluid or the corrosive-acetic mixture, it is seen that these early neuraxons appear frequently to approach, and to unite longitudinally with one another, thus giving to the nerve the structure of a coarse reticulum.

Among the other general stains tried Brazilin and Delafield's haematoxylin gave the most satisfactory results. Golgi impregnation and *intra-vitam* staining with methylen-blue were attempted, but repeated trials failed to produce the desired effect on the eye-muscle nerves of the embryos.

The method of van Gieson was used in advanced embryos for the purpose of studying the first stages in the formation of the sheaths of Schwann. By following Heidenhain's iron haematoxylin with the van Gieson mixture of acid fuchsin and picric acid, a good plasma stain was obtained, which brought out distinctly the cytoplasmic processes of the cells accompanying the nerve fibres, as well as those of the mesodermal cells.

Serial sections of embryos of various ages were made in the following planes: —

1. Parasagittal. Such series gave nearly longitudinal sections of the third and sixth nerves, and were best adapted to the study of their roots and the cell migration from the neural tube, since the roots of these nerves are spread out in a longitudinal but not in a transverse direction. Obliquely longitudinal sections of the ophthalmic branch of the fifth nerve were obtained in the series cut in parasagittal planes.

2. Transverse to the longitudinal axis of the mid-brain. Owing to the cephalic flexure, cutting in this plane gave longitudinal sections of the third nerve, obliquely longitudinal sections of the sixth, and nearly transverse sections of the ophthalmic branch of the fifth.

3. Frontal to the mid-brain; resulting in transverse sections of the third nerve, obliquely transverse sections of the sixth, and nearly longitudinal sections of the ophthalmic branch of the fifth.

## 2. Development of the Oculomotor Nerve, the Ciliary Ganglion and the Abducent Nerve; described by Stages.

Over fifty series of sections were made from chick embryos of various ages between the sixtieth hour and the seventh day of incubation, the period during which the third and sixth nerves and the ciliary ganglion assume both their distinctive histological characters and the most of their adult anatomical relations. From these series have been selected, for the purposes of the descriptions which follow, those best illustrating the successive steps in the development of the nervous structures with which we are dealing. For convenience in description I have divided the first five days of this period into five stages, which will be described in chronological order.

### *Stage I.*

1. *Oculomotor Nerve.* The earliest indication of the origin of the oculomotor nerve was observed in an embryo of seventy-two and one-half hours' incubation. That the development of this embryo had been abnormally retarded cannot be doubted, since the third nerve in a more advanced stage was frequently met with in embryos of seventy hours and in one case in an embryo of sixty hours.

Parasagittal sections of the mid-brain at this stage show the thickness of its ventral wall to be about one-eighth the height of the neural canal. Entering into the composition of the medullary wall may be observed the elements first described by His and later studied in such detail by Schaper ('97). Near the internal limiting membrane lie numerous germinative cells in process of division, while the mantle layer, making up the greater part of the wall, is composed of ependymal cells which have assumed a supporting function by developing into a medullary framework, and of the products of the proliferating activity of the germinative cells, namely, indifferent cells, which later differentiate into either nervous or supporting elements. The structure of the medullary framework is not well brought out by the vom Rath method. Near the external limiting membrane there is a narrow zone free from nuclei, the marginal veil ("Randschleier"). In addition, at a distance of about 100 micra from the median plane on either side, is to be seen a small group of cells which the vom Rath stain renders distinguishable from the other elements of the medullary wall. Each cell shows, at one side of its nucleus, a variable amount of darkly colored cytoplasm, the characteristic feature of a neuroblast. Inasmuch as these neuroblasts

occupy the position of those from which, at a slightly more advanced stage, the neuraxons of the oculomotor nerve take their origin, and since there is, even at this stage, evidence of the outgrowth of their processes from the neural tube, it follows that each group of cells is to be looked upon as the developing nidulus of the oculomotor nerve of that side. The niduli, then, at the very beginning of their development, are in a decidedly ventral position.

The neuroblasts on the right side of the median plane are more closely grouped together than those on the left, and even under low powers of the microscope stand out clearly from the rest of the medullary wall because of their darkly staining cytoplasm. Each group lies close to the external limiting membrane, projecting into the region of the marginal veil. On the right, the external limiting membrane remains intact, no processes of the neuroblasts having forced their way through it. On the left (Plate 3, Fig. 8), however, the external limiting membrane has been ruptured, and a part of the substance of the marginal veil protrudes. In the parasagittal section of the ventral mid-brain wall shown in Figure 8 there are included only a few of the several neuroblasts making up the nidulus. The letters *n'bl.'* designate a neuroblast with its cytoplasm drawn out, and directed toward the break in the medullary wall. Near it lie two cells, with cytoplasm tending in the same direction. The neuroblast marked *n'bl.''* shows a well-defined cytoplasmic process, narrowing toward its peripheral extremity, which has evidently pushed its way through the external limiting membrane. Of interest is a nucleus (*cl. med. mig.*), plainly medullary, lying in the material which is escaping from the neural wall through the aperture in the external limiting membrane to which reference has been made. This medullary nucleus appears to be making its way out of the neural tube at a very early stage in the development of the oculomotor nerve.

A little posterior to the oculomotor nidulus, at this stage, a few of the first fibres of the ventral fibre-tract can be seen running caudad toward the hind-brain.

2. *Eye Muscles.* As early as this stage, the fundament of the posterior rectus muscle has made its appearance, but since the series representing Stage II is more favorable for its study, description is deferred until then. Its character and relations in Stage I have not been altered in Stage II.

*Stage II.*

This stage is found in a series of seventy hours' incubation.

1. *Oculomotor Nerve.* Cross-sections of the mid-brain show the oculo-

motor niduli, lying in the ventral mid-brain wall, with their centres about 120 micra from the median plain. The niduli invade the marginal veil, which is now well developed. From the neuroblasts, cytoplasmic processes run out into the mesenchyme, as may be seen in Plate **3**, Figure 9. These appear to blend more or less, and, outside the neural tube, make a network with many nuclei lying along the threads of the net. This reticulated appearance of the nerve during its early development always follows fixation in Zenker's fluid or the corrosive-acetic mixture. In the present instance, the material was fixed in Zenker's fluid, and stained with Brazilin. Vom Rath preparations, on the contrary, exhibit the neuraxons of the growing nerve as separate elements, approximately parallel to one another, and not connected to form a network (comp. Plate **6**, Fig. 20).

The cells lying along the nerve strands are composed of rounded nuclei with which very little cytoplasm appears to be associated. For the sake of convenience, these cells will hereafter be called accompanying cells. Their nuclei resemble closely in form, size and staining qualities, the nuclei of the indifferent cells which lie inside the neural tube. Certain of the indifferent cells of the nidulus may be seen in the process of division (Plate **3**, Fig. 9, *cl.'*), and many lie near, and indeed in some cases (*cl.''*) immediately in contact with, the external limiting membrane.

The oculomotor may now be traced for some distance by its cytoplasmic threads and "accompanying" cells. It pursues a straight course through the mesenchyme in a ventral direction from the mid-brain. On account of the cephalic flexure, it lies nearly parallel to the axis of the hind-brain. After proceeding a short distance, it terminates in a slightly expanded distal extremity.

2. *Ophthalmic Branch of the Trigeminal Nerve.* In this stage the Gasserian ganglion, which has of course been present since its differentiation, much earlier, from the neural crest, shows plainly a partial division into a mesocephalic ganglion, giving rise to the ophthalmic branch of the trigeminus, and the ganglion of the maxillary and mandibular branches. The mesocephalic division is directed cephalad, and from its distal extremity the ophthalmic branch proceeds for a short distance along the outer wall of the anterior cardinal vein in the direction of the eyeball, but cannot be traced as far as the level of that organ. The maxillomandibular division of the Gasserian ganglion is directed ventrad and laterad, making approximately a right angle with the mesocephalic portion. It extends as far as the ectoderm, with a thickened patch of which (Plate **7**, Fig. 23, *gn. mx-md. Gas.*) it is directly connected.

The maxillary and mandibular branches of the trigeminus are poorly developed at this stage.

3. *Eye Muscles.* An isolated and compact accumulation of cells, staining deeply with haematoxylin, lies in the mesenchyme along the inner border of the anterior cardinal vein, not far from the ventro-lateral face of the anterior portion of the hind-brain (Fig. 23, *mu. rt. p.*). The distal extremity of the second division of the Gasserian ganglion lies laterad to this cell group, separated from it by the lumen of the anterior cardinal vein. The subsequent development of the mass proves it to be the fundament of the posterior rectus eye-muscle. No other eye-muscle fundaments are present, and not even the nidulus of the abducent nerve, which later innervates the posterior rectus muscle, has as yet appeared.

*Stage III.*

This stage is represented by two series of preparations, one of eighty-eight hours', the other of ninety-three hours', incubation. The two embryos had attained practically the same degree of development.

1. *Oculomotor Nerve and Ciliary Ganglion.* A fortunate section from the eighty-eight-hours' series, taken transversely to the axis of the mid-brain, shows the oculomotor cut longitudinally throughout its whole length (Plate 7, Fig. 24, *n. oc'mot.*). On either side of the median plane, and lying in the ventral wall of the mid-brain, the oculomotor niduli (*nidl. oc'mot.*) are seen as elliptical groups of cells, delimited by narrow areas comparatively free from medullary elements. These niduli present an elliptical outline whether viewed in transverse or parasagittal sections, but the ellipse seen in the latter plane has the longer principal diameter. Each nidulus measures approximately as follows: longitudinal diameter, 450 micra; transverse diameter, 135 micra; vertical diameter, 78 micra, the latter being more than half the thickness of the mid-brain wall, which here measures 117 micra. The centres of these niduli are slightly over 150 micra from the median plane. Allowing for increase in size due to growth, it is apparent that little, if any, change has taken place in their positions since their first appearance in Stage I. They then lay at the distance of 100 micra from the median plane.

The marginal veil is now invaded by the developing ventral fibre-tract, which forms a comparatively broad band of separation between the ordinary cells of the medullary wall and the external limiting membrane. The cells of the nidulus, however, project into this region, and extend to the very margin of the wall. Not all the neuraxons given off by the neuroblasts of the oculomotor nidulus pass out into the root of the

nerve. Some are directed caudad, and contribute to the formation of the ventral fibre tract, as can easily be observed in vom Rath preparations (Plate 6, Fig. 20, *trt. fbr. v.*). No fibres were observed running cephalad from the nidulus.

The nerve, consisting of fibres densely crowded with nuclei, arises by a root which is spread out in a longitudinal direction into a fan-shaped form (Fig. 20). Seen in a plane at right angles to this, the root does not show this enlargement (Plate 7, Fig. 24). The trunk of the nerve pursues a straight course ventrad and laterad, terminating at the side of the ventral extremity of the infundibulum (which is brought by the cephalic flexure into a position ventral to the mid-brain). The distal end of the oculomotor is conspicuously enlarged, so that the whole nerve, seen in cross-sections of the mid-brain, may be said to have a clavate form (Fig. 24, *n. oc'mot.*) In parasagittal sections, the enlargement has the shape of an unsymmetrical spindle, the posterior side of which has the sharper curvature. This terminal swelling is the fundament of the ciliary ganglion (*gn. cil.*).

Examination of the root of the oculomotor nerve with high powers reveals conditions strongly suggesting the migration of medullary cells. Figure 11 (Plate 4) represents a section through the root of the third nerve shown in Figure 24 (Plate 7). In this preparation, which was fixed in the corrosive-acetic mixture and stained with Heidenhain's iron haematoxylin, the processes projecting out from the neuroblasts run together and blend in such a way as to lose their identities as neuraxons. Again, as in Stage II, we find nuclei lying on the nerve fibres, both within and without the brain wall. Not all of those within are the nuclei of neuroblasts, for some (as *cl.'*) are without cytoplasmic processes, and others appear to be lying peripherally on processes apparently originating from neuroblasts more centrally placed. These nuclei, with which very little cytoplasm is connected, answer to the description of the indifferent cells of Schaper, which are known to possess the power of locomotion, since, within the boundaries of the medullary wall, they pass from the region of the germinative cells, where they originate, into the mantle layer. In the present instance they can be traced beyond the mantle layer to the free surface of the neural tube. Indeed, now and then, one can be detected in the position of *cl.''* lying half within and half without the neural wall, in the same position in which Dohrn ('91) has figured emigrating cells in the root of the developing oculomotor nerve in selachians. Although there is little evidence of cell division within the nidulus, the germinative cells at the inner border of

the medullary wall are numerous, and actively engaged in the production of indifferent cells.

Along the course of the nerve the "accompanying" cells may frequently be seen in process of mitosis, so that in case these are, as I believe, medullary and not mesodermal elements, it does not follow that the whole number present at this time have migrated from the neural tube, since their numbers are constantly increasing through cell division. A vom Rath preparation of the root of the oculomotor, in an embryo ninety-three hours old, shows a distinct difference in staining qualities between the "accompanying" nuclei and the adjacent mesodermal nuclei. While the "accompanying" nuclei take the stain readily, and are sharply defined, the surrounding mesodermal nuclei are, owing to their paleness, much less conspicuous (Plate **6**, Fig. 20). On the other hand, the nuclei of the medullary wall show in their affinity for the stain a striking resemblance to the "accompanying" nuclei.

It will be noticed that both the nuclei within the neural tube and those lying in the root of the oculomotor are more or less rounded in form. At least few, if any, exhibit a pronounced elongation. However, as we pass distally along the nerve, we find the most of the nuclei becoming more and more elongated, until the great majority are distinctly spindle-shaped. A few cells, distributed along the entire length of the nerve, retain the rounded form.

Taking into consideration the whole course of the nerve, the greatest amount of cell division occurs in the enlarged terminal (distal) portion (Plate **5**, Fig. 15), the fate of which proves it to be the fundament of the ciliary ganglion. Here, the majority of the nuclei are not as elongated as those lying among the fibres of the trunk of the nerve, many showing approximately circular outlines. All are nearly destitute of cytoplasm at this stage. It is evidently owing to the proliferation of these nuclei lying among the terminal nerve fibres that the enlargement in this region has taken place.

2. *Ophthalmic Branch of the Trigeminal Nerve.* The first branch of the fifth nerve (Plate **7**, Fig. 24, Plate **4**, Fig. 12, *rm. opth. trig.*) passes, in this stage, straight cephalad from the mesocephalic ganglion along the lateral wall of the anterior cardinal vein (*vn. crd. a.*). It terminates dorsad of the optic stalk between the fore-brain and eyeball, and just caudad of the laterally projecting vesicle of the hemisphere of that side. Its distal extremity is marked by a transitory fusiform ganglion (Plate **4**, Fig. 12, *gn. t'i.*), having an approximate length of 165 micra, and a greatest diameter of 75 micra. This Figure is drawn from several

sagittal sections of a vom Rath series, and represents about one-half the length of the ophthalmic branch of the trigeminus. In this particular case, the nerve, just after passing the level of the optic stalk, divides into two branches, which unite again at the ganglion; but I do not find this condition to be a constant one.

An examination of longitudinal sections of the ophthalmic branch of the trigeminus at this stage shows that, beside the elongated "accompanying" cells, resembling those of the oculomotor, there are to be found distributed along the whole length of the nerve, as well as in the transitory ganglion, ganglionic cells whose larger nuclei and more abundant and deeply staining cytoplasm make them easily distinguishable from the "accompanying" cells. The description of these ganglionic cells, and the discussion of the terminal ganglion, will be taken up under Stage IV.

In the transverse series of an eighty-eight-hours' chick there can be seen at this stage, on the left side of the body, an exceedingly slender offshoot of the ophthalmic branch of the trigeminus, passing to the fundament of the ciliary ganglion. The offshoot is apparently composed of a single neuraxon, to which a few "accompanying" cells are applied, and at the place of its origin from the ophthalmic branch lies a cluster of ganglion cells. On the right side of the body, on the contrary, the most careful search has failed to reveal any fibrous connection whatever with the fundament of the ciliary ganglion. Of interest, however, are two cells having the appearance of ganglion cells, both of which lie in the mesenchyme between the ophthalmic branch and the fundament of the ciliary ganglion, one being about midway between the two structures, and the other close to the surface of the ganglionic fundament. Both these cells differ markedly from mesodermal cells, and also from those of the fundament of the ciliary ganglion, on account of the larger size of their nuclei and their deeply staining cytoplasm; while their resemblance to ganglion cells seen in the ophthalmic branch, in the same sections, is very close. They appear to be migrating ganglion cells, and their origin from the ophthalmic branch seems very probable. Further evidence that ganglion cells do migrate from this nerve will be presented in Stage IV.

3. *Abducent Nerve.* The sixth nerve was first met with in a seventy-eight-hours' series, that is, in a stage intermediate between Stages II and III. It was observed on both sides of the head as a very small nerve, arising by five delicate roots from the ventral surface of the hind-brain, about 135 micra from the median plane. The nerve can be traced

cephalad for a very short distance only. In another series of the same length of incubation the abducens was not found. It will be noted that the abducens appears several hours after the differentiation of the fundament of its muscle, the posterior rectus.

In the ninety-three-hours' series, the nidulus of the abducens can be seen lying close to the ventral surface of the hind-brain wall. It is elongated in a longitudinal direction, and lies about 175 micra from the median plane. It is not possible, in my preparations, to make out its limits as definitely as in the case of the oculomotor nidulus.

The nerve itself is a slender one, springing from the ventral face of the hind-brain by a varying number of attenuated roots, placed one behind the other, and crowded with "accompanying" cells. As many as eight roots have been counted, the number differing in different embryos, and even on opposites sides of the body in the same embryo. The roots unite a short distance from the brain to form the trunk of the nerve, which passes straight cephalad, running parallel to the ventral face of the hind-brain. In vom Rath preparations the nerve can easily be traced as a slender bundle of a few darkly stained neuraxons with elongated "accompanying" cells to the posterior edge of the compact cluster of cells making up the fundament of the posterior rectus muscle. It will thus be seen that the abducens, though appearing later than the oculomotor, is the first of the two nerves to become connected with a muscle fundament. The fundament of this muscle, the posterior rectus, was already recognizable in the mesenchyme of Stage I, therefore, long before any of the other eye-muscle fundaments, none of which can be made out previous to the present stage.

4. *Eye Muscles.* The fundament of the posterior rectus eye muscle lies near the anterior portion of the hind-brain in a ventro-lateral position. It consists of a mass of modified mesodermal cells, which differ from surrounding ones in their closer association and their rather greater amount of cytoplasm. The absence of fibres in the muscle fundament, and their presence in that of the ciliary ganglion, make a notable difference in the appearance of these two structures. The muscle mass is elongated in an antero-posterior direction (Plate **7**, Fig. 23, *mu. rt. p.*). It lies mostly caudad of the fundament of the ciliary ganglion, but its anterior end runs cephalad and laterad as a narrow prolongation, which, passing laterad of the posterior half of the ganglionic fundament, terminates between it and the anterior cardinal vein. The fundament of this muscle appears in Plate **7**, Figure 24 (*mu. rt. p.*), where the section has passed very near its anterior extremity.

The fundaments of the dorsal rectus and dorsal oblique muscles, and the common fundament of the anterior and ventral rectus muscles, are now to be seen. These consist of small, local differentiations of mesodermal cells, which tend to become more closely associated, and, through their activities, produce an abundance of cytoplasmic material; this material represents the first stage in the formation of the contractile substance of the muscle cells. These muscle fundaments will be described and figured in Stage V, where my sections are in planes more favorable for showing their relations. Their positions in the later stage are practically the same as in the present one.

The fundament of the ventral oblique muscle cannot be distinguished at this stage.

It might here be stated that it has been impossible, in the chick, to assign the eye-muscle fundaments to their respective somites by the aid of the head cavities. These have not been present in the stages studied owing to the fact that, in the chick, they appear to be obliterated very early in development — much earlier than is the case in the embryos of ducks (van Wijhe, '86; Rex, :00) terns, gulls and lapwings (van Wijhe, '86).

*Stage IV.*

This stage occurs at about the one hundredth hour of incubation. It is described from two series, one of one hundred hours, the other of one hundred and one hours.

1. *Oculomotor Nerve.* The oculomotor nidulus retains its extreme ventro-median position. In fact it appears to have moved toward rather than away from the median plane. Although the neural tube has increased in size, the centre of the nidulus now lies nearly 50 micra nearer the median plane than in the stage last described, being distant only 105 micra from it. The ventral fibre tract has increased in thickness, and, while the nidulus of the oculomotor extends into it, the lower border of the nidulus does not lie as near the external limiting membrane as in Stage III. Neuraxons from the nidulus, passing through the ventral fibre tract — a region free from nuclei — are frequently seen to be accompanied by nuclei which have the rounded form of the indifferent elements in the nidulus dorsal to them, and of the "accompanying" cells of the root of the oculomotor ventral to them. Farther out on the trunk of the nerve, the great majority of the cells have become elongated, though occasional round ones are to be observed.

The oculomotor nerve pursues, as in the stage last described, a

straight course, passing ventrad and slightly laterad (Plate 4, Fig. 13) through the mesenchyme, between the infundibulum on its median, and the lower half of the eyeball on its lateral side. The large accumulation of cells forming the ciliary ganglion lies mainly on the lateral, or ocular, side of the nerve trunk (Compare Plate 7, Fig. 25 *gn. cil.*). The neuraxons of the nerve continue for a short distance beyond the ganglion, and, bending somewhat laterad, terminate immediately mediad of the antero-ventral portion of the eyeball in the fundament of the ventral oblique muscle, which makes its first appearance in this stage. The oculomotor is as yet entirely without branches.

A histological change has by this time taken place in, at least, the distal two-thirds of the nerve. In early stages, especially in vom Rath preparations, the neuraxons of the nerve are to be seen, under high powers of the microscope, as relatively thick fibres with peripherally situated "accompanying" cells. The same powers now show, especially in the more distal parts of the nerve, that the relatively thick fibres no longer appear, their places having been taken by much finer fibrils. As a consequence, the fibrous components of the nerve are now greatly increased in number without a corresponding increase in the calibre of the nerve. I shall hereafter speak of these fine filaments as *fibrils* in contradistinction to the earlier *fibres* — the coarser structures which the fibrils have replaced. The histogenesis of the fibrils is considered under Stage V.

Lying at all depths within the nerve, "accompanying" cells may be seen closely applied to the fibrils. In the description of the preceding stage, evidence was brought forward to show that these "accompanying" cells have been derived through migration from the neural tube, where, it is maintained, they originate as rounded, indifferent cells, the descendants of the germinative cells, and where, according to Schaper, they may later differentiate into either nervous or supporting elements, i. e., neuroblasts or spongioblasts. Certain of these cells, through their power of locomotion, are capable of leaving the central nervous system, and, following the path of the neuraxons, of reaching the peripheral nerve trunk. Lying among the fibrils of the nerve, they increase in number by division. Knowing, as we do, the subsequent history of the indifferent cells remaining within the neural tube, an analogous fate might be expected, *a priori*, in the case of those which migrate out into the nerve trunk. That many of these emigrant indifferent cells do eventually subserve a supporting function, not in the form of neuroglia, but as the sheaths of Schwann, can hardly be doubted. Such cells become elongated soon

after their escape from the medullary wall, and, during succeeding stages of development, can be observed adhering closely to the nerve fibrils, until finally, just before the hatching of the animal, they give evidence of participation in the formation of the sheaths of Schwann. The other possibility indicated by their identity with the indifferent cells of the neural tube — namely, their differentiation, in part, into ganglion cells — will be considered under the subject of the ciliary ganglion.

In the one-hundred-hours' series there occurs on the oculomotor nerve midway between its root and the ciliary ganglion, a group of cells worthy of attention. As is shown in Plate 5, Figure 17 — which is a longitudinal section through a portion of the oculomotor nerve — these cells are placed at the margin of the nerve trunk, and present a striking contrast to the "accompanying" cells lying along the fibrils. While the nuclei of the latter cells are drawn out into oval, elliptical and spindle forms, and possess very little cytoplasm, those of the cells forming the group have mostly an almost circular outline, and lie embedded in an abundance of granular cytoplasmic material, which stains deeply with haematoxylin. Proximal and distal to this group the nerve exhibits only fibrils and the ordinary elongated "accompanying" cells. I shall have occasion to refer again to this accumulation of differentiated cells when discussing the ciliary ganglion. The single layer of strikingly elongated cells at the periphery of the nerve is probably made up of mesodermal elements. The processes of these cells unite to form a thin envelope, which doubtless represents the connective-tissue sheath or perineurium of the adult nerve trunk.

2. *Ophthalmic Branch of the Trigeminal Nerve.* The transitory ganglion which appeared in Stage III still persists, but in one series it presents on the left side of the body a disorganized appearance, being represented by small clumps of ganglion cells, which lie scattered about in the mesenchyme in the immediate vicinity of the place on the ophthalmic branch where one would expect to find the ganglion. Possibly we have here the beginning of a process of disintegration. The ganglion on the right nerve in the same series is as compact and definitely limited a body as in the preceding stage, and it lies in the same position, immediately posterior to the laterally projecting vesicles of the cerebral hemispheres. The nerve continues beyond the ganglion as a slender strand, which runs ventrad of the lateral vesicle of the fore-brain, trending also laterad as it proceeds, and, before reaching the level of the anterior extremity of the fore-brain, terminates in a small swelling containing ganglion cells. Several small and rather indefinite

branches are given off immediately proximal to the large transitory ganglion. These branches can be followed for short distances into the mesenchyme.

As was stated in the description of Stage III, ganglion cells can be found scattered along the nerve from the mesocephalic to the transitory ganglion. These cells are more numerous near the mesocephalic ganglion; in fact, the transition from ganglion to nerve is a very gradual one, since so many ganglion cells have migrated outside the true limits of the ganglion. Comparison shows that the ganglion cells to be found in the mesocephalic ganglion are precisely like those of the transitory ganglion. Both have relatively large, rounded nuclei containing chromatin which is concentrated, for the most part, into one or two large masses. Surrounding the nucleus, but lying mostly on one side of it, is a considerable amount of finely granular cytoplasm, which becomes drawn out to a blunt extremity, and stains a deep blue with iron haematoxylin. The nucleus itself is much less deeply stained, with the exception of the included chromatin particles, which take on a dense, black appearance. Such ganglion cells are shown in Plate **2**, Figure 5, where $\alpha$ and $\beta$ are cells from the transitory ganglion and $\gamma$, a cell from the mesocephalic ganglion. These ganglion cells are plainly in an early stage of development, being in the condition of neuroblasts the cytoplasm of which has become drawn out to one side preparatory to developing into neuraxons. In the mesocephalic ganglion many ganglion cells have already sent out their neuraxons, and these form the trunk of the ophthalmic division of the fifth nerve. There are also present, however, young ganglion cells which have not reached this stage of development, as the one to which attention has just been called (Fig. 5, $\gamma$). In fact, such ganglion cells seem to be forming here through the activities of proliferating cells, which can be seen in every section made through the Gasserian ganglion in this and preceding stages. Since, then, the mesocephalic ganglion is the scene of constant cell production, and since its young ganglion cells resemble exactly those of the transitory ganglion, and are connected with them by a continuous series of similar ganglion cells lying scattered along the nerve, the source of the component cells of the transitory ganglion does not seem to be open to question, especially when we remember that young ganglion cells possess an extraordinary capacity for locomotion (His, Jun., '**91**). All the evidence points to a migration of ganglion cells distally along the nerve to form the transitory ganglion and, similarly, any other smaller ganglia that may be found near it.

If we look upon the proliferating cells in the mesocephalic ganglion as comparable, to a certain extent, with the germinative cells of the neural tube, and consider them, as Schaper has proved for the germinative cells, the producers of a generation of indifferent cells capable of becoming in part nervous, in part supporting elements, then we can easily account for the presence in the ganglion of many small, rounded cells, almost destitute of cytoplasm. It seems probable that these correspond to the indifferent cells of the neural tube. While some of them may change to ganglion cells, others may develop into the small, somewhat elliptical cells found in the ganglion, especially near its distal end, where they pass by an easy gradation into the more elongate cells lying among the fibrils of the nerve which here takes its origin. This leads to the supposition that the "accompanying" cells of the nerve, which later subserve a supporting function by developing the sheaths of Schwann, have been derived through migration from the ganglion. In support of this view I have introduced a drawing (Plate 2, Fig. 6) made from sections of an embryo of Amblystoma punctatum. Group *A* is taken from the central part of the Gasserian ganglion. It will be noticed that here, lying within the limits of the ganglion, are to be found nuclei in all stages of transition between the rounded and the elongated forms. The nuclei of group *B* lie on the neuraxons at the proximal end of the ophthalmic branch of the trigeminal nerve, those designated by *cl. comit.'* and *cl. comit.''* being situated at the emergence of the nerve from the ganglion. The oval one (*cl. comit.'*) I take to represent a stage in the differentiation of a rounded cell into a long "accompanying" cell, such as those to be found in the remainder of the course of the nerve. Neither in the chick nor in Amblystoma is there at any point along the ophthalmic branch evidence of an intrusion of mesodermal cells.

It is quite possible that supporting derivatives of indifferent cells remain in the ganglia, and later, through their activities, form the nucleated capsules of the ganglion cells, just as the "accompanying" cells of the peripheral nerve form, in an analogous manner, the sheaths of Schwann about the neuraxons. The similar origin of the capsule of the ganglion cell and of the Schwann's sheath of its process would account for the continuity of the two structures. Those investigators who affirm the mesodermal derivation of the Schwann's-sheath cells have never been able, so far as I know, to obtain evidence of an invasion of cerebro-spinal ganglia by mesodermal elements destined to give rise to the envelopes of the ganglion cells.

The rôle played by the ophthalmic branch of the trigeminus in the development of the ciliary ganglion will be considered under the following heading.

3. *Ciliary Ganglion.* As has been stated, the ciliary ganglion lies mainly on the lateral side of the third nerve near its distal extremity. Its form in transverse section, and its general relation to the ophthalmic branch of the fifth nerve and to the eyeball, are shown in a diagrammatic way, for the right side of the head, in Plate 4, Figure 13. The striated portion represents the fibrils of the oculomotor, while the ciliary ganglion is indicated by the evenly shaded part. The ganglion contains, now for the first time, cells so far advanced in differentiation that they can be declared without hesitation to be young ganglion cells (Plate 5, Fig. 16, *E*). Interspersed among them are a few cells (*E*, *cl.'*, *cl.''*) exactly like the "accompanying" cells of the nerve fibrils. The ganglion is no longer, as in the preceding stage, the scene of active cell division. A few of the young ganglion cells lie along the median border of the nerve opposite the laterally placed body of the ganglion. Figure 13 (Plate 4) also shows the ophthalmic branch of the trigeminus (*rm. opth. trig.*) cut transversely. A small offshoot is indicated, running from this nerve in the direction of the ciliary ganglion, which, however, it fails to reach. The fibrils of this branch become lost in the mesenchyme, so that it is not possible to trace them all the way to the ganglion, although, on the opposite side of the head, where development is a little more advanced, this can be done. I have also indicated in the figure all the ganglion cells to be found along that segment of the ophthalmic branch of the trigeminus which lies opposite the ciliary ganglion as well as all the ganglion cells that have become detached from the ophthalmic branch at this level. The number and positions of these cells were ascertained by studying the series of consecutive sections extending from the anterior to the posterior face of the ciliary ganglion, and recording the ganglion cells observed by projecting them on the plane of the diagrammatic section represented by Figure 13.

A comparison of the ganglion cells of the ophthalmic branch of the trigeminus with those of the ciliary ganglion will prove instructive. In Figure 16 at *A* are shown two ganglion cells ($\alpha$, $\beta$), taken from the ophthalmic branch, their positions in that nerve being indicated by the same letters in Figure 13. The ganglion cells of the group *E* were taken at random from the ciliary ganglion. They exhibit the features characteristic of young ganglion cells, resembling those of the ophthalmic branch in the possession of deeply staining granular cytoplasm, accumu-

lated at one side of the rounded nucleus. But an evident and consistent difference exists between the cells taken from the two sources. The cells of the ciliary ganglion are smaller than those of the ophthalmic branch of the trigeminus. This difference is to be seen at once in the drawings in Figure 16, in which the outlines of the cells were made with the aid of the camera lucida under precisely the same optical conditions for all groups. Not only is the amount of cytoplasm less in the ciliary-ganglion cells, but their nuclei are distinctly smaller than those of the ophthalmic cells. It is not an easy matter to compare accurately the two classes of cells by measurements of the diameters of their respective nuclei, since these are seldom exactly circular in outline. I have, however, made measurements in the cases of such nuclei as approached nearest to a circular form, and I find that while the diameters of the nuclei of the ciliary-ganglion cells fall between 5.2 micra and 6.5 micra, the nuclei of the ophthalmic cells show constantly a diameter of approximately 7.8 micra.

The ganglion cells lying in the mesenchyme, between the ophthalmic branch and the ciliary ganglion, are very evidently emigrant ophthalmic cells. Two such cells, having the positions $\gamma$ and $\delta$ in Figure 13, are shown in *B* and *C*, Figure 16, each surrounded by mesodermal cells. A glance shows that they belong to the ophthalmic and not to the ciliary type. Within the boundaries of the ciliary ganglion, lying close to the exterior of the cell mass, on the side toward the ophthalmic branch of the trigeminus, are to be found among the smaller ganglion cells three cells with large nuclei, two of which are shown in Figures 16, *D*, and 13 ($\epsilon$, in both figures). These appear to be ophthalmic ganglion cells, which have traversed the mesenchyme and entered the ciliary ganglion.

With the foregoing evidence before us, let us inquire into the source of the cells of the ciliary ganglion. We have seen that in the early stages of the growth of the oculomotor nerve a migration of medullary cells takes place from the neural tube into its root. I believe that in Stage I we have the first migratory cell forcing its way through the external limiting membrane of the neural tube (Plate 3, Fig. 8). In succeeding stages these cells seem to be migrating in considerable numbers. The rounded nuclei of the cells appear to be almost naked, for it is difficult to detect any cytoplasm surrounding them. These cells are as yet neither neuroblasts nor spongioblasts, but evidently the motile, indifferent cells of Schaper, i. e., they are descended from germinative cells, and are capable of differentiating later into either nervous

or supporting elements. Many of these nuclei, once out on the nerve, become elongated as they move away from the neural tube. Such "accompanying" cells maintain throughout development their close proximity to the nerve fibrils, and in them we recognize, as has been pointed out, the nuclei of the future sheaths of Schwann. A large part, then, of the emigrant cells become supporting elements.

Do any of the emigrant indifferent cells become nervous elements? We have seen that at all stages rounded nuclei, resembling and continuous with the indifferent cells of the neural tube, occur abundantly at the root, and more sparingly along the trunk, of the oculomotor nerve, lying among the more numerous elongated supporting cells. In Stage III an accumulation of such cells was observed at the distal end of the nerve, causing at this place its enlargement into the fundament of the ciliary ganglion (Plate **7**, Fig. 24, *gn. cil.*), the cells of which were undergoing active division (Plate **5**, Fig. 15). Schaper, it will be remembered, shows that the indifferent cells of the central nervous system likewise possess the property of further propagation. In the present stage, IV, the ciliary ganglion of the right side contains undoubted ganglion cells. The right oculomotor has not yet come into connection with any other nerve, although the ophthalmic branch of the fifth is sending fibrils in the direction of the ciliary ganglion, and toward the latter a few ophthalmic ganglion cells are apparently making their way through the mesenchyme. Three, in fact, lie just within the borders of the ciliary ganglion, easily distinguishable by their larger size from the numberless ganglion cells about them. The vast majority of the cells of the ciliary ganglion, however, could have originated only by differentiation from the rounded, proliferating cells which are to be seen in Stage III occupying the site of the future ganglion, before there is the slightest trace either of a connection between the oculomotor and the ophthalmic branch of the trigeminus, or of the migration of ophthalmic ganglion cells through the mesenchyme toward the fundament of the ciliary ganglion. There is good evidence that the actively dividing cells of the latter ganglion had their origin in indifferent medullary cells which had escaped from the neural tube. If this be so, then a small portion of the indifferent cells migrating out from the mid-brain do become differentiated, after increase in numbers by division, into nervous elements, i. e., ganglion cells of the ciliary ganglion.

An accumulation of differentiating cells at the side of the third nerve, midway between its root and the ciliary ganglion (Plate **5**, Fig. 17), during this stage, has been referred to in the account of the oculomotor

nerve. This accumulation I believe to be composed of young ganglion cells, developing from indifferent cells stranded, as it were, on their way from the neural tube to the ciliary ganglion. Around each nucleus the characteristic deeply staining cytoplasm is in process of development. Whether these cells later disintegrate, or produce secondary ganglia, such as are sometimes found in fishes, amphibians, birds and mammals (Schwalbe, '79; Jegorow, '86–87), I do not know. Such an accumulation does not occur along the nerve of the opposite side.

4. *Abducent Nerve.* The sixth nerve emerges in this stage, as in the preceding, by several roots, crowded with "accompanying" cells. Its nidulus is not well defined. The roots of the nerve lie about 190 micra from the median plane, and have much the appearance seen in the following stage, which is shown in Figure 18 (Plate 6). They unite ventral to the hind-brain to form a straight, unbranched nerve, which runs horizontally cephalad, becoming more attenuated toward its distal end, and terminating in the postero-dorsal extremity of the posterior rectus muscle.

In the roots of the nerve can be seen evidence of cell migration from the neural tube. The more or less rounded cells found at the proximal end of the nerve pass rapidly into the elongated "accompanying" cells, which lie distally along its fibrils. Many of the "accompanying" cells show mitotic figures. At no place on the abducens is there an accumulation of rounded cells like that which in Stage III forms on the oculomotor the fundament of the ciliary ganglion. In the case of the sixth nerve the indifferent cells which migrate out from the hind-brain appear to develop exclusively into structures with the supporting function, the sheaths of Schwann.

5. *Eye Muscles.* In this stage first appears the fundament of the ventral oblique muscle, the most anterior of the four eye muscles which are innervated by the oculomotor nerve. At the same time we find that the free end of the nerve extends beyond the ciliary ganglion as far as this muscle mass.

The axis of the posterior rectus muscle now points in a postero-dorsal and antero-ventral direction, instead of nearly longitudinally, as in Stage III. The dorsal, anterior and ventral rectus muscles retain their primitive relations in both this stage and the following one; in the account of the latter they are described and figured, together with the ventral oblique muscle.

### *Stage V.*

The conditions characteristic of the fifth stage of development are found in embryos of one hundred and eighteen to one hundred and

twenty hours. The cephalic flexure has become less pronounced, so that the axis of the fore-brain makes with that of the hind-brain approximately a right angle, at the vertex of which the large vesicle of the mid-brain projects externally.

1. *Oculomotor Nerve.* The oculomotor nidulus is now about 200 micra from the median plane. The fibre tract of the ventral wall of the mid-brain is well developed, forming a third of the entire thickness of the wall. The processes of the oculomotor neuroblasts extend ventrad through this tract, the fibres of which run at right angles to the processes. Along the oculomotor neuraxons lie "accompanying" cells, which resemble in every respect the cells which are present in great abundance in the root of the nerve immediately outside the external limiting membrane.

However, within the neural tube the "accompanying" cells do not often extend along the neuraxons all the way to the limiting membrane. It appears that migration is by this time nearly over. The neuraxons of the third nerve are closely interwoven with numerous nerve fibres running at right angles to them. The "accompanying" cells, which measure approximately 4 micra in diameter, in order to pass from the mantle layer to the mesenchyme, would be obliged to force their way along the oculomotor neuraxons through a feltwork about 75 micra in thickness.

From its root, which is still spread out longitudinally, the oculomotor nerve runs caudad, ventrad and somewhat laterad. On reaching the level of the optic stalk, it turns toward the median plane, and, continuing its course ventrad and mediad for a short distance, ends in the ventral oblique eye muscle. About two-thirds of the way from the proximal to the distal end of the nerve, the unsymmetrically elliptical ciliary ganglion is to be seen lying on the nerve trunk. This portion of the nerve is seen in the parasagittal section shown in Plate **7**, Figure 26 (*n. oc'mot.*), which is viewed from the right face. The fundament of the dorsal rectus eye muscle (*mu. rt. d.*) and the common fundament of the ventral and anterior rectus muscles (*mu. rt. v.* + *a.*) lie in close contact with the dorsal side of the nerve, but no fibrils can be detected turning aside from the main trunk to penetrate these muscle masses. That is to say, so far as can be seen, the oculomotor remains at this stage an unbranched nerve.

Along the posterior margin of the root occurs a bundle of fibres rendered conspicuous by their freedom from "accompanying" cells. Along the external margin of this bundle a single layer of cells separates it from the mesenchyme.

A study of the oculomotor with high powers at this stage gives evidence of different histological conditions at different points along the nerve. These conditions are well brought out by the vom Rath method. A heavy black precipitate along the neuraxons differentiates these clearly against the less darkly colored stroma in which they appear to be imbedded.

The drawings shown in Plate 2, Figure 7, *A*, *B*, *C*, are from a chick of one hundred eighteen hours' incubation, stained by the vom Rath method. These drawings were made from different parts of the nerve: *A*, from the proximal end, *B*, farther distally, and *C*, near the peripheral termination. All were made with the aid of a camera lucida under precisely the same conditions of magnification. At *A*, are shown two neuraxons taken from the root as they appear under the $\frac{1}{12}$" oil-immersion lens. It will be seen that these are compact cylinders in which fibrillation cannot be very satisfactorily made out, although indications of it are to be detected here and there, for instance, in the upper portion of the neuraxon lying on the right in the drawing. Closely applied to the neuraxons are numerous elongated "accompanying" cells.

Farther out on the nerve, in that portion which passes along the ventral margin of the common fundament of the ventral and anterior rectus muscles (comp. Plate 7, Fig. 26), the conditions are different. From this part of the nerve I have drawn two bundles of fibrils with the accompanying embryonic nuclei of Schwann's sheath. These possibly represent two neuraxons, though it is impossible to trace any one neuraxon with certainty through the series of sections from the root of the nerve to this place. A careful study of the preparations has, however, convinced me that the compact neuraxon which passes out from the brain into the root of the nerve becomes more and more expanded and more evidently fibrillar toward its peripheral extremity. In fact, close to this extremity the fibrils become so numerous that those belonging to the various component neuraxons of the nerve are inextricably intermingled. The appearance of the oculomotor upon reaching the ventral oblique muscle is shown at *C*, Figure 7. The bundles of fibrils which are the continuations of the comparatively large cylindrical neuraxons of the proximal portion of the nerve have here, at its distal end, lost their identity in the mass of fibrillar elements. Nevertheless, while the separate bundles of fibrils corresponding to the neuraxons are not well defined, there can be seen in the nerve a tendency toward the formation of more or less well-marked groups of fibrils, each of which may represent in the main a single neuraxon.

That the number of the fibrils arising from the splitting up of neuraxons is increased distally along the nerve by longitudinal division of the fibrils, can be asserted with a fair degree of confidence. It is possible to see in the preparations strong indications of such branching, though, as the fibrils concerned are very fine and lie closely associated with one another, it is difficult in most cases, even with the highest powers and most careful focusing, to be perfectly sure that the two branches into which the fibril appears to divide are really the continuations of that fibril.

In preparations fixed in Zenker's fluid and stained with iron haematoxylin the finely fibrillar condition of the entire nerve is plainly demonstrated, for not only are the identities of the neuraxons lost in the mass of fibrils in the distal and middle parts of the nerve, but they are with difficulty made out close to the root, where the fibrillar condition also appears.

It is interesting to compare with the conditions in the chick longitudinal sections through the oculomotor nerve of a pig embryo measuring 8 mm. (its greatest length in its normal curved position). This material was fixed in the corrosive-acetic mixture mentioned on p. 176 and stained with Brazilin. In Plate **4**, Figure 14, *A*, the horizontal line (*mb. lim. ex.*) represents the external limiting membrane of the midbrain, and above it, on its way through the ventral fibre tract, is seen the proximal end of an oculomotor neuraxon. Upon leaving the neural tube, the neuraxon gradually grows thicker as it proceeds peripherally, and its fibrillation becomes more marked. It can be followed as a distinct process as far as has been indicated in the drawing, but at this point it comes into relation with other neuraxons forming the root of the oculomotor, and its identity becomes lost. The lower drawing, *B*, is that of a longitudinal section through the same nerve about midway in its course. It is plain that here the separate neuraxons cannot be distinguished. The whole nerve is simply a homogeneous bundle of fibrils.

A noticeable difference between the pig and the chick is the almost complete absence of nuclei among the nerve fibrils of the former. A few cells are to be seen lying along the periphery of the nerve, but it is only occasionally that one can be found among the fibrils in the interior of the bundle.

2. *Ophthalmic Branch of the Trigeminal Nerve.* The Gasserian ganglion is still bifurcated distally, but the two parts are more completely united proximally than in earlier stages. The ophthalmic branch extends forward from the extremity of the tapering mesocephalic ganglion, passes just mediad of the anterior cardinal vein, and dorsad of

the fundament of the posterior rectus muscle. Then the nerve, upon reaching the region of the ciliary ganglion, which is ventral to it, sends to this ganglion a strong fibrillar communicating branch (Plate 7, Figure 25, parasagittal section, *rm. comm.*), indications of which were present in the preceding stage. The ophthalmic branch in its course next passes immediately ventral to the median end of the dorsal rectus muscle; then, passing on the dorsal side of the distal end of the optic stalk, and keeping close to the eyeball, it runs to the anterior region of the head, where it breaks up into a number of slender branches.

Ganglia are not to be seen along either the right or the left ophthalmic branches, although ganglion cells occur here and there along their courses. The ganglionic swellings observable in Stage IV have completely disappeared. At only two points on each nerve are there accumulations of ganglion cells, and these are so small as to cause no enlargement of the nerve trunk. The ganglionic groups occur at the origin of the communicating branch (Fig. 25, $\beta$), and at the origin of a small ramus which extends into the mesenchyme opposite the dorsal rectus muscle. Both of these localities are far proximad of that of the transitory ganglion of Stages III and IV, no trace of which now remains. It will be remembered that in Stage IV one of the two ophthalmic nerves showed a disorganized transitory ganglion, which, as was suggested, might be considered in process of disintegration.

In a series from a chick incubated one hundred nineteen and one-half hours, the communicating branch between the ophthalmic division of the trigeminus and the ciliary ganglion is of considerable size, its diameter on the right side of the body, where it is a single trunk, being rather more than one-fourth that of the ophthalmic branch. On the left side, it consists of two separate bundles of fibrils. In both cases it presents the same appearance as the trunk of the ophthalmic branch, being made up of fine fibrils with elongated "accompanying" cells. The very small number of migrant ganglion cells to be found along the communicating branch is significant. Instead of affording a highway along which quantities of cells from the ophthalmic branch pass over into the third nerve to form the ciliary ganglion, it serves for the transit of very few of these cells. On the left side of the body there can be counted along the communicating branch only five ganglion cells, while, on the right side, only one undoubted cell of this nature can be made out. In the trunk of the ophthalmic branch, at the place of origin of the communicating ramus, there are present, on the left side of the body, twenty-one ganglion cells; on the right side, only two.

3. *Ciliary Ganglion.* The ciliary ganglion projects as a large mass of cells from the lateral and dorsal sides of that portion of the oculomotor nerve which lies between the fundament of the dorsal rectus muscle and the combined fundaments of the ventral and anterior rectus muscles (Plate 7, Fig. 26, *gn. cil.*). The communicating ramus from the ophthalmic branch of the trigeminus connects with its lateral face. A comparison of its ganglion cells with those found along the ophthalmic branch shows, as in Stage IV, a marked difference both in the size of nuclei and in the amount of distinctive cytoplasm. In Plate 3, Figure 10, the three upper ganglion cells, $\alpha$, $\beta$, $\gamma$, are from the ophthalmic branch, the three lower ones, $\delta$, from the ciliary ganglion. Cell $\alpha$, near which are shown several future Schwann's-sheath cells applied to the neuraxons, is taken from the nerve close to the Gasserian ganglion. Cell $\beta$ is found at the base of the communicating branch running to the ciliary ganglion, in the position indicated in Plate 7, Figure 25, by $\beta$; while cell $\gamma$ occurs distal to $\beta$ at the point indicated by $\gamma$ in the same figure. The smaller size of the ciliary ganglion cells, $\delta$, is apparent. Both along the ophthalmic branch and within the ciliary ganglion, occasional "accompanying" cells can be observed in the act of dividing. One from the ophthalmic branch is shown at *cl. comit.'*, one from the ciliary ganglion at *cl. comit.''*

Ganglion cells of the ophthalmic type lying along the communicating branch have been mentioned above. In one case ophthalmic ganglion cells can be traced along the branch to the boundary of the ciliary ganglion, but none can be detected within it; in another, there can be seen within the boundaries of the ganglion near the entrance of the communicating branch two cells, which can with certainty be assigned to the ophthalmic class. It is apparent that there is not a large contribution of cells from this source to the ciliary ganglion; yet it seems probable, in view of the evidence derived from Stage IV, that at least a few ophthalmic ganglion cells have from that time on been making their way into the ciliary ganglion. If this be so, the large invading cells must soon become modified, through decrease in size, into cells closely resembling those of the ciliary ganglion, which have arisen *in situ*, for careful search through the entire ganglion at this stage fails to reveal more than two cells of the ophthalmic type within its precincts, and these lie close to the entering end of the communicating branch, and hence appear to be new arrivals.

4. *Abducent Nerve.* Except for some increase in size, the sixth nerve has not changed its appearance since the last stage. It springs from its

longitudinally elongated nidulus in the floor of the hind-brain by five or more roots placed one behind the other (Plate 6, Fig. 18, *n. abd.*). These roots often become divided near their region of emergence from the neural tube, so that a strict count of their number is made difficult. The number of roots differs in different individuals, and even on opposite sides of the same individual. Although Figure 18 shows but five roots, on the other side of the embryo six can be counted. This figure shows the manner in which the roots unite to form the nerve, which is now of comparatively large size, although previously it has been remarkable for its small calibre. The nerve trunk (Plate 7, Fig. 26, *n. abd.*) runs cephalad, laterad, and slightly ventrad to end in the fundament of the posterior rectus muscle (*mu. rt. p.*). In one case, the nerve bifurcates just before reaching the fundament, and while one division continues the course of the nerve straight into the muscle mass, the other runs on the dorsal side of it for some distance, and then turning ventrad enters the fundament, dividing again as it does so. The fibrils of the nerve can be traced far into the mass of differentiated mesodermal cells constituting the muscle fundament.

The evidence for cell migration into the roots of the abducens is still good at this stage, the ventral fibre tract in the region of the nidulus being less thick than where the oculomotor emerges. Figure 19 (Plate 6), drawn from a preparation fixed in the picro-sulphuric mixture and stained with Delafield's hæmatoxylin, shows that rounded nuclei in the wall of the neural tube extend down from the nidulus into the ventral fibre tract, and, indeed, to the very surface of the tube, where they become continuous with similar nuclei lying outside the tube along the nerve. Farther out on the nerve these nuclei become elongated. As has been said, all the indifferent cells escaping in connection with the sixth nerve appear to assume a supporting function, developing into the sheaths of Schwann. At no time during the growth of the nerve can young ganglion cells be discovered at any place along its course.

5. *Eye Muscles.* The section represented in Plate 7, Figure 26, shows in one plane the fundaments of the posterior rectus eye muscle and the four eye muscles innervated by the oculomotor nerve. All the eye-muscle fundaments consist of clusters of crowded elongated mesodermal nuclei, the interstices between which are filled with differentiating cytoplasm, which stains deeply with hæmatoxylin.

The first, in order of development, is the posterior rectus muscle (*mu. rt. p.*). This was present in Stage I, and its position and appear-

ance during succeeding stages have been described. In the present stage, it lies caudad and mediad of the eyeball. The main mass of the muscle extends laterad until its outer extremity comes to lie just ventrad of the ophthalmic branch of the trigeminus (Plate **7**, Fig. 25, *mu. rt. p.*). From this main portion an arm extends mediad and caudad to meet the abducent nerve; the nerve penetrates the muscle mass, and its fibrils become thoroughly intermingled with the cells of the fundament.

The fundaments of the dorsal, ventral, and anterior rectus and the dorsal oblique muscles were all seen for the first time in Stage III. The dorsal rectus (Plate **7**, Fig. 26, *mu. rt. d.*) extends in a transverse direction, its outer end lying dorsad of the ophthalmic branch of the trigeminus, and its inner end dorsad of, and in close contact with, the oculomotor, immediately before that nerve reaches the ciliary ganglion.

The common fundament of the ventral and anterior rectus muscles (*mu. rt. v.* + *a.*) is comparatively large, and lies along the ventral side of the optic stalk, reaching laterad as far as the third nerve, which lies along its ventral border.

The most superficial of all the muscle fundaments is that of the dorsal oblique, which lies in the same parasagittal plane as the Gasserian ganglion, and is dorsal to the eyeball at a point a little anterior to one opposite the choroidal fissure. This is the only eye-muscle fundament which does not appear in the plane of the section of which Figure 26 is a photograph. It has no connection with its nerve, the trochlear, which, indeed, I have not been able to detect up to this stage of development. In appearing thus before its nerve, it resembles the posterior rectus muscle, and differs from the muscles innervated by the oculomotor.

The last muscle to develop is the ventral oblique (Fig. 26, *mu. ob. r.*), which first appeared in Stage IV. Its fundament is antero-ventral to the optic stalk, and much nearer the median plane than the dorsal oblique. The axis of the muscle fundament extends transversely, and near its median end receives the spreading fibrils of the extremity of the oculomotor nerve. It is the only one of the muscles finally innervated by the third nerve into which fibrils from the nerve can at the present time be traced.

### *Notes on Later Development.*

1. *Oculomotor Nerve.* In Stage V (about five days' incubation) none of the branches of the third nerve found in the adult were present. In series of about seven days' incubation it can be seen that the eye-muscle fundaments, which in Stage V were in contact with the sides of the

nerve, have now drawn away from it. Fibrils can be detected turning aside from the trunk of the oculomotor and entering the fundament of the dorsal rectus. The nerve also sends a bundle of fibres to the fundament of the ventral rectus, which has now become distinct from that of the anterior rectus. The branch of the oculomotor which in the adult innervates the latter muscle must develop later, since I have not been able to detect it in three series of sections of about seven days' incubation. At this time, then, the third nerve exhibits all of the branches found in the adult, with the single exception of that to the anterior rectus muscle.

The first indications of the formation of the sheaths of Schwann were discovered only in the oldest embryo I have examined — one of eighteen days' incubation. In longitudinal sections of the oculomotor stained by the van Gieson method, it is apparent that the elliptical "accompanying" cells lying among and parallel to the fibrils are sending out cytoplasmic processes from both ends. These processes have not, however, developed the longitudinal laminae, which mark the next step in the formation of the sheaths of Schwann in mammals, as was first shown by Gurwitsch (:00). Bardeen (:03) using the van Gieson stain, has confirmed the conclusions of Gurwitsch. When present the laminae, exhibiting their cut edges in cross-sections of the nerve fibrils, separate the latter into bundles which are later bound closely together to form the neuraxons. Cross-sections of the oculomotor nerve at this stage give no evidence of the subdivision of the fibrils into such bundles.

2. *Ophthalmic Branch of the Trigeminal Nerve.* The direct connection between the ophthalmic branch and the ciliary ganglion persists at least up to the eighteenth day of incubation, which is the oldest series in which I have looked for it. It will be remembered that in the adult such a direct connection does not exist, the communicating branch entering the ciliary nerve some distance distal to the ciliary ganglion. (Plate 1, Fig. 2).

3. *Ciliary Ganglion.* The ciliary ganglion retains throughout life its situation immediately on the trunk of the oculomotor nerve; that is to say, no radix brevis is ever developed. In fact it often appears, even in the adult, as though the main trunk of the oculomotor ends in the ciliary ganglion, while its continuation to the ventral oblique muscle has the appearance of a large branch leaving the trunk just before the ciliary ganglion is reached. It is, however, more in accordance with the facts of comparative anatomy to regard the nerve as dividing into two main branches at the region of the ramus to the dorsal rectus muscle.

One of these main branches is the latter ramus, the one innervating the dorsal rectus muscle; the other is the ventral ramus, which extends to the ventral oblique muscle. The ciliary ganglion should, then, be considered as sessile on the ventral division of the nerve.

At seven days I have seen no evidence of the ciliary nerve. At eighteen days it is present as a comparatively large bundle of fibrils, which runs from the ciliary ganglion to the eyeball. The fibrils penetrate the sclerotic coat caudad of the entrance of the optic nerve, and continue their course between the sclerotic and choroid tunics.

In the seven-days' stage the difference in size between the ganglion cells of the ciliary and Gasserian ganglia is striking.

In the ciliary ganglion of an eighteen-days' embryo, small cells with crescentic nuclei can be seen arranged about the ganglion cells. Their processes are continuous, forming a complete envelope, the nucleated capsule of the ganglion cell. Retzius ('81) figures these nucleated capsules around the bipolar ciliary-ganglion cells of the adult fowl.

4. *Abducent Nerve.* The sixth nerve had reached in Stage V its adult condition, as far as its relations to the posterior rectus muscle and surrounding structures were concerned, except that its branch to the muscles of the nictitating membrane had not appeared. I have not observed the formation of this branch. It could not be found at the end of seven days' incubation.

5. *Eye Muscles.* As has been said in the description of the oculomotor nerve, at some period between Stage V (about one hundred nineteen hours) and one hundred sixty-eight hours, the common fundament of the ventral and anterior rectus muscles becomes divided into two parts, from which develop the separate muscles of the adult.

6. *Trochlear Nerve.* In sections of seven-days' embryos, the fourth nerve can be traced from its dorsal superficial origin between the midbrain and the hind-brain to its termination in the dorsal oblique muscle. Before reaching the muscle the nerve spreads out into a loose brush of fine fibrils, which, even in vom Rath preparations, are not easily traceable through the mesenchyme.

## Discussion of Results.

*Migration of Medullary Cells.* Frequent allusions to the migration of cells from the embryonic neural tube are to be found in neurological literature since the time of Balfour. That pioneer among the investigators of the histogenesis of nerves believed he found in elasmobranchs clear evidences of the escape of nerve-forming cells from the spinal cord

(Balfour, '75). In this view he was supported by Beard ('88). Dohrn ('88[a], '91) also saw the migration of medullary elements in selachians, and C. L. Herrick ('93) affirms that in amphibians, reptiles and mammals nerve-forming cells issue from the nuduli of ventral nerve roots. Platt ('96) is of the opinion that in Necturus the motor nerves are formed by emigrant bipolar cells derived from the neural tube. Among recent observers who have likewise seen medullary cells migrating out into the ventral roots of spinal nerves may be mentioned Harrison (:01) and Neal (:03). The former made his observations upon Salmo salar, the latter upon Squalus acanthias.

The interpretations put upon these emigrant cells have been various. They have been thought by some observers to be the cells which, assuming a moniliform arrangement, give rise to the neuraxons of the nerves; they have been considered to be ectodermal additions to the mesodermal cells which lie among the peripheral processes of centrally located neuroblasts, and to be destined later to participate in the formation of the sheath of Schwann; and, finally, they have been looked upon, in part at least, as undeveloped ganglion cells.

It is not unusual to meet with ganglion cells in the ventral roots of the spinal nerves of adult animals. Freud ('78) describes such cells in the ventral roots of the spinal nerves of Petromyzon. Schäfer ('81) and Kölliker ('94[a]) found ganglion cells similarly situated in the cat. Thompsen ('87) figures what he believes to be ganglion cells in various stages of degeneration in the roots of the third and fourth cranial nerves in man, and his interpretation has been accepted by Gaskell ('89), and more recently by Barratt (:01), to account for the presence of amorphous fibrillar or granular masses observed by them in the human oculomotor and trochlear nerves. Taking into consideration the observations which have been made on the growth of the oculomotor nerve in the chick, the suggestion is here offered that these degenerated ganglion cells may have had their origin as indifferent cells migrating out from the mid-brain into the roots of these nerves in an early embryonic stage. Becoming differentiated here into ganglion cells, they may later have undergone a more or less complete disintegration, owing to their failure to attain to a condition of functional activity.

The chief opponent of the view that medullary cells migrate out from the neural tube has been W. His (see His, '89), and it is doubtless largely owing to his prestige that the fact of migration, together with the rôle played by the emigrant cells in nerve formation, has attracted comparatively little attention. The followers of His have accounted for

the origin of all the parts of a peripheral neuron in the following manner: The neuraxon arises as a process from a centrally situated neuroblast, which becomes the ganglion cell. Cells of mesodermal origin form around the neuraxon the sheath of Schwann (Vignal, '83; Kolster, '99; Gurwitsch, :00; Bardeen, :03; and others). Between the central neuraxon and the enveloping sheath of Schwann the non-cellular medullary substance is developed, possibly as the result of differentiation in the homogeneous stroma immediately surrounding the neuraxon. The participation of emigrant medullary elements in the formation of the nerves appears, then, to be quite unnecessary.

Recent writers on the histogenesis of nerves generally take into account, however, the indisputable evidence that has been offered in favor of the immigration of cells into the developing nerves of elasmobranchs. Bardeen (:03) admits that even in mammals, as well as in the lower vertebrates, a certain number of cells migrate out from the spinal ganglia and cord, but believes that in mammals the cells of Schwann's sheath arise in the main at least from the mesoderm. Neal (:03) is of the opinion that in elasmobranchs both mesodermal and emigrant ectodermal cells participate in the production of the sheath of Schwann. He saw few mitoses among the emigrant cells, and consequently concludes that ectodermal elements are not present in sufficient numbers to furnish all the sheath cells. In the developing oculomotor and abducent nerves in chick embryos however, mitotic division among the "accompanying" cells is of frequent occurrence. If, as the appearances lead me to believe, the "accompanying" cells are medullary derivatives, it follows that here ectodermal elements might easily be multiplied until they became numerically equal to the nuclei of the Schwann's sheaths of the definitive nerve. Proof that the Schwann's-sheath cells of the lateral line in Amblystoma are to be regarded as ectodermal derivatives has been adduced by Harrison (:03) in a recent paper.

In an earlier paper Neal (:00) concluded from his own researches, and from a review of the literature, that anamniote embryos differ from amniote embryos in deriving a part of their sheath cells from the neural tube. That migration is more easily observable in lower forms than in mammals is undoubtedly true. In this connection it should be borne in mind that His ('89), who controverted the interpretations of Dohrn ('88) in respect to migration in selachians, must have been largely influenced by his extended researches on the histogenesis of mammalian nerves. But if it be admitted that medullary cells migrate out of the neural tube into the roots of the third and sixth nerves in the chick,

Neal's limitation of such a phenomenon to anamniote embryos no longer holds.

*Histogenesis of the Neuraxons.* As regards the growth and internal differentiation of the neuraxons, my results are in general accordance with those of Vignal ('83), and especially with those of Bardeen (:03), both of whom followed the development of cerebro-spinal nerves in mammalian embryos. The neuroblasts of the oculomotor nidulus send out into the mesenchyme, to form the third nerve, compact homogeneous processes of considerable calibre. These soon break up, apparently by a longitudinal splitting, into fine fibrils; this differentiation begins at the distal end of the nerve. Bardeen's statement as to the effect of this upon the appearance of a cerebro-spinal mammalian nerve applies equally well to the oculomotor or abducent nerve in the chick. He says (p. 247): "During the early stages of development these fibrils may either be gathered in small compact groups, each of which represents an axis-cylinder process, or they may be so scattered within the nerve that it is impossible to distinguish definite groups of fibrils corresponding to axis-cylinder processes." The first statement we have seen to be true of the middle portion of the developing oculomotor nerve; the second, of its distal extremity. Bardeen is also of opinion that the fibrils may increase in numbers by longitudinal division. As he has pointed out, these fibrils seem to be of larger calibre than the "primitive" fibrils of Apáthy and Bethe occurring in adult neuraxons. According to the observations of Vignal ('83), Gurwitsch (:00) and Bardeen the embryonic nerve trunk in mammals becomes invaded by cells which divide the fibrils into small bundles, each of which becomes surrounded by a special envelope (Schwann's sheath) formed by the invading cells. The fibrils making up the bundle are closely bound together in this way into a compact neuraxon. It is possible that the fibrillation of the adult neuraxon may bear some relation to the embryonic fibrils out of which the neuraxon is formed, but the precise nature of this relation remains to be ascertained.

*Nature of the Ciliary Ganglion.* The evidence derived from the study of both its development and its histology points to the double nature of the ciliary ganglion of the fowl. I shall now consider, first from an embryological, and then from a histological point of view, the character of the two kinds of elements that enter into the composition of the ganglion.

We have seen that during development the fundament of the ciliary ganglion receives contributions of cells from the Gasserian ganglion *via*

the ophthalmic branch of the fifth nerve. Their origin from a cerebro-spinal ganglion, their migration, their final incorporation into a ganglion whence proceed motor peripheral fibres, are all strong indications of the sympathetic nature of these cells. Yet, as His ('88[a]) has observed, errant cerebro-spinal cells, which may remain cerebro-spinal in character throughout life, cannot with certainty be distinguished in the embryo from cells destined to become sympathetic. We must apply in addition the tests of histology after the cells have reached their adult development.

The greater part of the cells of the ciliary ganglion do not originate, like those to which reference has just been made, from a cerebro-spinal ganglion. It has been shown that a large proportion of the ciliary cells become gradually differentiated *in situ* in the third nerve. The evidence in favor of regarding these as migrant medullary elements has been given. These ganglion cells, therefore, resemble in their development neither cerebro-spinal cells, which stand in direct genetic relation to the neural crest, nor sympathetic cells, the derivation of which from cerebro-spinal ganglia can scarcely be questioned since the researches of Onodi ('86), His, Jun. ('91), and others.

The histological conditions which obtain in the ciliary ganglion of the adult fowl bear out well the idea of its double nature. The occurrence in the ganglion of two regions, which were described in Part I of this paper, is readily explained on developmental grounds. The smaller, dorsal portion of the ganglion, whose small cells, slightly medullated neuraxons and pericellular fibrils give evidence of its sympathetic character, lies on the side next the ophthalmic branch of the trigeminus, and receives from that nerve the communicating ramus which so clearly resembles a ramus communicans of the thoracic region. It is true, however, that the neuraxons given off peripherally lack the abundant medullation characteristic of the post-ganglionic sympathetic neuraxons of the thoracic region. If we consider that this part of the ganglion has originated from those cells which, in the embryo, migrated into it from the Gasserian ganglion, it then may be said to conform to the sympathetic type in its manner of development, as well as in nearly all its adult histological details. One evidence of the sympathetic nature of this region of the ciliary ganglion has not been adduced. The multipolarity of its cells still remains a matter of assumption. Holtzmann ('96), it is true, found both large and small cells in the ciliary ganglion of birds. While the large cells are shown to be bipolar with large, medullated processes, a typical small cell is figured with a single, slender, apparently

non-medullated process. The possibility that other fine processes might have been torn off in the macerating and teasing method employed suggests itself. Except for its unipolar condition this cell bears a close resemblance to a sympathetic cell.

The larger, ventral region of the ciliary ganglion bears little resemblance to a sympathetic ganglion. The cells in it are of greater size, pericellular fibrils are not abundant, and its neuraxons are heavily medullated. It seems safe to assume that from this portion of the ganglion were obtained by maceration the large bipolar cells with medullated processes, the description of which has been given. While the neurons in question resemble in medullation and size of ganglion cells those of cerebro-spinal ganglia, some points of difference exist, the chief one being the bipolarity of the former and the unipolarity of the latter. As has been stated, even when the ciliary-ganglion cells approach an unipolar condition, the single stem does not divide at right angles to form the typical T of a cerebro-spinal neuron. Inasmuch as we have assigned to the dorsal or sympathetic portion of the ciliary ganglion the cells which in the embryo migrate into it from the Gasserian ganglion, this larger ventral region must have had its origin from those other and more numerous cells which differentiate into nervous elements *in situ* within the third nerve. These, as has been shown, appear to be migrant cells from the ventral wall of the neural tube, a source from which neither cerebro-spinal nor sympathetic ganglion cells are derived.

It must be borne in mind, however, that the definition of an embryonic sympathetic cell which is here implied, namely, a ganglion cell which migrates out from a "stationary" cerebro-spinal ganglion into a "vagrant" ganglion (Gaskell), may be found by later researches to be inadequate. If later researches prove Harrison (:01) to be right in his supposition that sympathetic ganglia receive contributions of migrant motor cells from the neural tube *via* the ventral roots of spinal nerves, then the two sources of the cells in the ciliary ganglion become identical with the two sources of sympathetic cells. But the participation of medullary elements in the formation of sympathetic ganglia has never been actually observed. Investigators such as Onodi ('86), His, Jun., und Romberg ('90), and His, Jun. ('91), who have made special studies of the development of the sympathetic system, agree in deriving the cells entirely from spinal ganglia. Their conclusions have been generally accepted.

The ciliary ganglion of the fowl is, then, to be considered as composed of cells which fall into two categories, one being, as far as the evidence goes, in all essential respects typically sympathetic, the other belong-

ing to neither the sympathetic nor cerebro-spinal systems. This conception of the double nature of the ciliary ganglion differs from that of Krause ('81) who, on anatomical grounds, regards the ganglion in mammals as mainly sympathetic, but also in part cerebro-spinal. He looks upon the ganglion in vertebrates lower than mammals as a homologue of a spinal ganglion. But in the light of the foregoing observations on its development in the chick, the direct genetic connection of a portion of the ciliary ganglion with the neural crest in vertebrates appears open to question. No embryologist who has distinguished between the ciliary and the mesocephalic ganglia has ascribed to any of the cells of the former a cerebro-spinal origin.

In birds, as we have seen, only a small proportion of the ciliary cells enter the ganglion from the ophthalmic branch of the trigeminal nerve. But if Hoffmann ('85) has described correctly the development of the ciliary ganglion in Lacerta, it is evident that in reptiles, at least, the greater part of the cells are derived by migration from the Gasserian ganglion. In view of this fact the large number of ganglion cells which, in chick embryos, migrate out along the ophthalmic branch of the fifth nerve, but do not take part in the building of the ciliary ganglion, may possibly possess a phylogenetic significance; for it is difficult to account for the migration of these short-lived cells into the ophthalmic branch, except upon the supposition that the cells were of functional importance earlier in the history of the race. The majority now fail to reach the ciliary ganglion, and soon disappear. It seems possible that a change in the relative numbers of ciliary cells from the two sources, the Gasserian ganglion and the neural tube, may have been in some way connected with the striking development in birds of the intrinsic muscles of the eye, to which the ciliary nerves are distributed. It is characteristic of the group that these muscles are striated. The radial dilator muscle of the iris is remarkably well developed. The latter organ appears to be under voluntary control (Coues, :03).

Rubashkin (:03) has recently shown that in the chick certain cells derived from the Gasserian ganglion form, at the extremity of a ramus of the ophthalmic branch of the fifth nerve, a "ganglion olfactorius nervi trigemini." According to his account, these cells do not leave the Gasserian ganglion before the seventh day of incubation. They are consequently not connected with the cells which, much earlier, migrate out along the ophthalmic branch during the development of the fundament of the ciliary ganglion. Many of these, as has been shown, are grouped together in one or more transitory ganglia, which are observable at the

ninety-third and at the one-hundredth hour. By the end of the fifth day, the transitory ganglia and the majority of the cells scattered along the nerve have disappeared.

*Homologies of the Oculomotor and Abducent Nerves.* A consideration of the homologies of the third and sixth nerves does not lie within the scope of this paper. Nevertheless, I have thought it well to reproduce here from Fürbringer (:02, p. 124) a concise summary of the various interpretations given these nerves and their musculature. I have slightly modified the arrangement.

I. Oculomotor Nerve.

1. Ventral-motor nerve (somatic musculature).
   a. Based on ontogeny: van Wijhe ('82), Beard ('85), Hoffmann ('85, '94, '96, '97, '99, :00), His ('88), Martin ('90, motor [sensory ?] root in addition), Dohrn ('90, represents in Torpedo a multiplum equivalent to from three to four, if not more, spinal nerves, '91), Zimmermann ('91), Kölliker ('96), Neal, ('96, '98).
   b. Based on anatomy in adult: Bell ('30), Stannius ('49), Huxley ('74–75), Schneider ('79), Gaskell ('86, '89), Strong ('90) Fürbringer ('97), Wiedersheim ('98), Gaupp ('97–99).
2. Ventral-motor nerve (visceral musculature).
   a. Based on ontogeny: von Kupffer ('94 ?, '95).
   b. Based on histology of eye muscles: Stannius ('51), Langerhans ('73).
3. Lateral-motor nerve (visceral musculature).
   a. Based on ontogeny: Balfour ('78), Marshall ('81, '82), Dohrn ('85, '87), Houssay ('90), Hatschek ('92, probably descended from the visceral constrictions), Sewertzoff ('98–99, not entirely decided).
4. Arising as a dorso-sensory or mixed (dorso-sensory and motor) nerve, with secondary reduction of the sensory component.
   a. Based on ontogeny: Marshall ('82), possibly Rabl ('89), Martin ('90), Platt ('91), Mitrophanow ('92, '93), Sedgwick ('94), perhaps Sewertzoff ('98–99).
   b. Based on comparative anatomy: Schwalbe ('79, '81), Gaskell ('89), [Haller ('98)].

II. Abducent Nerve.

1. Ventral-motor nerve (somatic muscle).
   a. Based on ontogeny: van Wijhe ('82), Beard ('85), His ('88), Dohrn ('88, '90, p. 344: represents in Torpedo a multiplum equivalent to three or four motor spinal nerves), Martin ('90), Oppel ('90), Zimmermann ('91), Platt ('91), Hatschek ('92,

pro-otic myomere), Hoffmann ('94, '96, '97, '99, :00), von Kupffer ('94, p. 58 : probably a lateral body muscle, but derivation from a velar muscle not entirely disproved; '95, pp. 36, 72: m. rect. ext. of Petromyzontes identical with that of Gnathostomi), Sewertzoff ('95, '98–99, formed from one somite in sharks, from at least two in Torpedo), Kölliker ('96), Neal ('96, m. rect. ext. formed from four metameres).

b. Based on anatomy in adult: same as oculomotor nerve (I. 1. b.).

2. Lateral-motor nerve (visceral muscle).

a. Based on ontogeny: Balfour ('78), Marshall ('81), Dohrn ('85, p. 447: m. rect. ext. formerly a gill muscle), von Kupffer ('94, p. 58: derivation from the velum not disproved, though probably from a lateral body muscle).

b. Based on histology of external rectus muscle: Stannius ('51), Langerhans ('73). Schneider ('79) maintains the contrary [ventral motor nerve].

## Summary of Results.

### I. Oculomotor Nerve.

1. The oculomotor nerve of the chick first appears during the third day of incubation as a small bundle of peripheral processes of neuroblasts on either side of the median plane. These neuroblasts are grouped to form the oculomotor nidulus, which is situated in the ventral wall of the mid-brain near the median plane.

2. Rounded medullary cells (indifferent cells of Schaper) migrate from the neural wall into the root of the developing oculomotor nerve. During the peripheral growth of the neuraxons, these escaped medullary elements continue their migrations, and become distributed along the nerve as "accompanying" cells. In vom-Rath preparations the "accompanying" cells exhibit staining qualities which differentiate them from the neighboring cells of the mesenchyme.

3. The majority of the "accompanying" cells become elongated, and remain during development closely applied to the neuraxons. They are recognizable in later stages as the cells from which the sheaths of Schwann are derived. The sheaths of Schwann are consequently, like the other parts of the oculomotor neurons, ectodermal in origin. A small number of the "accompanying" cells retain their rounded form, and through increase in numbers by mitotic division give rise to a cluster of cells near the distal end of the oculomotor nerve. In this way the fundament of the ciliary ganglion is formed, many of the cells of the enlarged extremity of the nerve developing into ganglion cells.

4. The processes of the oculomotor neuroblasts grow out into the mesenchyme as compact neuraxons. After they have attained a considerable length they begin to break up, apparently by longitudinal splitting, into fine fibrils. The process of fibril formation seems to begin at the free extremity of the nerve and proceed toward the proximal end. As a consequence the whole nerve trunk, especially in its more distal regions, presents the appearance of a large bundle of fine fibrils, among which the fibrils belonging to individual neuraxons cannot with certainty be recognized.

5. The fundaments of the muscles to which the oculomotor nerve is distributed in the adult appear after the development of the nerve has begun. The last of these muscle fundaments to be differentiated, that of the ventral oblique muscle, is the first with which the third nerve becomes connected. The branches of the nerve to the dorsal and ventral rectus muscles are developed between the fifth and seventh days of incubation.

6. The oculomotor nerve in the adult fowl is composed of large and small medullated neuraxons. The majority of the small neuraxons form a peripheral layer along the lateral margin of the nerve, and are continued into the ciliary ganglion.

## II. Ciliary Ganglion.

1. The fundament of the ciliary ganglion appears during the fourth day as a collection of actively dividing "accompanying" cells near the distal extremity of the oculomotor nerve. There is evidence that these "accompanying" cells are to be regarded as indifferent medullary cells which have migrated into the nerve from the neural tube. During the fifth day nearly all the cells become differentiated into young ganglion cells, characterized by a comparatively large amount of deeply staining, granular cytoplasm, which is accumulated, for the most part, at one side of the rounded nucleus. A few of the indifferent cells do not become ganglionic nervous elements, but assume the characters of elongated supporting cells, similar to those which develop the sheaths of Schwann along the trunk of the nerve. These cells may later participate in the formation of the nucleated capsules of the ciliary-ganglion cells.

2. While the fundament of the ciliary ganglion is undergoing development, young ganglion cells migrate out from the Gasserian ganglion along the ophthalmic branch of the trigeminal nerve. These cells are characterized by the same deeply staining, granular cytoplasm and rounded eccentric nuclei observable in the ciliary cells, but they are

easily distinguished from the latter by reason of the larger size of their nuclei and the greater abundance of their cytoplasm. A comparatively small number of the ophthalmic ganglion cells migrate into the fundament of the ciliary ganglion, passing at first through the mesenchyme, and later along the neuraxons of a communicating ramus, which grows from the ophthalmic branch of the trigeminus to the fundament of the ciliary ganglion. In their origin from a cerebro-spinal ganglion, and in their capacity for locomotion, these cells resemble those which, in the trunk region, give rise to sympathetic ganglia. Of the ganglion cells migrating along the ophthalmic branch, those which fail to reach the ciliary ganglion are in part accumulated to form one or more transitory ganglia in the distal portion of the nerve. By the end of the fifth day the transitory ganglia, and nearly all the migrant ganglion cells distributed along the nerve, have disappeared.

3. In the adult fowl the ciliary ganglion is situated directly upon the oculomotor nerve without the intervention of a radix brevis. Two regions are distinguishable in the ganglion, a smaller dorsal region and a larger ventral one. The first region presents many sympathetic characters, containing, as it does, small ganglion cells, slightly medullated neuraxons and many pericellular fibrils. It receives neuraxons of small calibre through the communicating branch from the trigeminus. The communicating branch resembles histologically a ramus communicans of the sympathetic system. From this region of the ganglion practically non-medullated neuraxons proceed to the eyeball in company with the other neuraxons constituting the oculomotor ciliary nerve. This portion of the ciliary ganglion may have arisen from the migrant ophthalmic cells which enter the fundament of the ganglion during development. The larger ventral region of the ganglion contains large cells, fine but well-medullated neuraxons, and few pericellular fibrils. From this region are doubtless derived the large bipolar ganglion cells with medullated processes which are to be obtained from the ciliary ganglion by maceration methods. The greater part of the oculomotor ciliary nerve passes out from this portion of the ganglion in the form of a large bundle of fine medullated neuraxons. It is probable that the cells of this region had their origin in the migrant cells from the embryonic neural tube.

4. The oculomotor ciliary nerve passes from the distal end of the ciliary ganglion to the intrinsic muscles of the eye. It comprises fine, well-medullated neuraxons together with a small number of neuraxons with little if any medullation. In its course through the orbit it gives

rise to a variable number of very small branches, which accompany it to the eyeball.

### III. Abducent Nerve.

1. The sixth nerve arises at the beginning of the fourth day of incubation as a slender bundle of processes of neuroblasts, which emerge from the ventral face of the hind-brain. The abducent nidulus is situated in the ventral wall of the hind-brain near the median plane. The nerve exhibits a number of attenuated roots arranged in a longitudinal series.

2. Indifferent medullary cells migrate out into the roots of the abducent nerve. These distribute themselves along the nerve trunk, assume an elongated form, and become recognizable as the cells which later develop the sheaths of Schwann. None of these indifferent cells give rise to ganglion cells.

3. The fundament of the posterior rectus muscle becomes differentiated during the third day before the appearance of the abducent nerve. The embryonic abducent neuraxons, upon emerging from the neural tube, grow rapidly cephalad to connect with the muscle fundament.

4. The abducent nerve of the adult is composed of both large and small medullated neuraxons.

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1. Cells resembling the indifferent cells of the neural tube are present in the embryonic Gasserian ganglion. Certain of these cells may, by differentiation, give rise to ganglion cells. Others appear to migrate from the Gasserian ganglion into the ophthalmic branch of the trigeminal nerve, and there assume the characters of Schwann's-sheath cells. Similar cells remaining within the Gasserian ganglion possibly develop later into the nucleated capsules of the ganglion cells.

2. In the adult the communicating ramus from the ophthalmic branch of the trigeminus does not pass directly to the ciliary ganglion, as in the embryo, but to the oculomotor ciliary nerve, with which it connects about one mm. from the distal extremity of the ganglion. Certain of the neuraxons of the communicating ramus, however, turn centrally and enter the sympathetic region of the ciliary ganglion; the remaining neuraxons accompany those of the oculomotor ciliary nerve to the eyeball.

3. A trigeminal ciliary nerve is given off by the communicating ramus about midway in its course. A second trigeminal ciliary nerve is occasionally to be seen arising from the communicating ramus near the termination of the latter in the oculomotor ciliary nerve.

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## EXPLANATION OF PLATES.

All figures are from preparations of chick embryos except when otherwise stated. All, with the exception of the photomicrographs and diagrams, were drawn with the aid of the camera lucida. The magnifications follow the descriptions of the figures.

ABBREVIATIONS.

| | |
|---|---|
| *cl.* | Indifferent cell. |
| *cl.'* | Indifferent cell. (See explanation of Fig. 16.) |
| *cl.''* | Indifferent cell. (See explanation of Fig. 16.) |
| *cl. comit.* | "Accompanying" cell. |
| *cl. com't.'* | "Accompanying" cell. |
| *cl. comit.''* | "Accompanying" cell. |
| *cl. gn.* | Ganglion cell. |
| *cl. gn. cil.* | Ganglion cell of ciliary ganglion. |
| *cl. med. mig.* | Migrant medullary cell. |
| *ec'drm.* | Ectoderm. |
| *gn. cil.* | Ciliary ganglion. (See explanation of Fig. 17.) |
| *gn. cl.''* | Erroneously engraved for *gn. cil.* (Fig. 2). |
| *gn. Gas.* | Gasserian ganglion. |
| *gn. mx-md. Gas.* | Maxillo-mandibular portion of Gasserian ganglion. |
| *gn. t'i.* | Transitory ganglion. |
| *if'b.* | Infundibulum. |
| *l.* | Lateral. |
| *m.* | Median. |
| *mb. lim. ex.* | External limiting membrane. |
| *ms'e.* | Mid-brain. |
| *ms'ench.* | Mesenchyme. |
| *mt'e.* | Hind-brain. |
| *mu. ob. d.* | Dorsal oblique muscle. |
| *mu. ob. v.* | Ventral oblique muscle. |
| *mu. rt. a.* | Anterior rectus muscle. |
| *mu. rt. d.* | Dorsal rectus muscle. |
| *mu. rt. p.* | Posterior rectus muscle. |
| *mu. rt. v.* | Ventral rectus muscle. |
| *mu. rt. v. + a.* | Ventral and anterior rectus muscles. |
| *n. abd.* | Abducent nerve. |
| *n. cil. oc'mot.* | Oculomotor ciliary nerve. |
| *n. cil. trig.* | Trigeminal ciliary nerve. |
| *n. oc'mot.* | Oculomotor nerve. |

*n. opt.* . . . . . Optic nerve.
*n. trch.* . . . . . Trochlear nerve.
*n'ax.* . . . . . Neuraxon.
*n'ax. med.* . . . . Medullated neuraxon.
*n. b'l.* . . . . . Neuroblast.
*n. b'l.'* . . . . . Neuroblast.
*n. b'l.''* . . . . . Neuroblast.
*nd. Ranv.* . . . . . Node of Ranvier.
*nidl. oc'mot.* . . . . Oculomotor nidulus.
*nl.* . . . . . Nucleus.
*par. ms'e. v.* . . . . Ventral wall of mid-brain.
*par. mt'e. v.* . . . . Ventral wall of hind-brain.
*par. rtn. ex.* . . . . Outer wall of retina.
*par. rtn. i.* . . . . Inner wall of retina.
*pdl. opt.* . . . . . Optic peduncle.
*phx.* . . . . . Pharynx.
*pi'n.* . . . . . Perineurium.
*prose.* . . . . . Fore-brain.
*rm. comn.* . . . . Communicating ramus.
*rm. f.* . . . . . Frontal branch.
*rm. mu. qd. + pyr.* . Branch to quadratus and pyramidalis muscles.
*rm. mu. rt. a.* . . . Branch to anterior rectus muscle.
*rm. mu. rt. d.* . . . Branch to dorsal rectus muscle.
*rm. mu. rt. p.* . . . Branch to posterior rectus muscle.
*rm. mu. rt. v.* . . . Branch to ventral rectus muscle.
*rm. na.* . . . . . Nasal branch.
*rm. n. cil. oc'mot.* . . Branch from oculomotor ciliary nerve.
*rm. opth. trig.* . . . Ophthalmic branch of trigeminal nerve.
*rm. v.* . . . . . Ventral ramus.
*trt. fbr. v.* . . . . Ventral fibre tract.
*vel. marg.* . . . . Marginal veil.
*vn. crd. a.* . . . . Anterior cardinal vein.

## PLATE 1.

Fig. 1. Eye-muscle nerves of adult fowl viewed from left side. × 4.

Fig. 2. Portion of Figure 1 enlarged. × 16.

Note. — The dotted line from *rm. comn.* is carried a little too far.

mu.rt.d.
mu.ob.d.
rm.comm.
n.cil.oc'mot.
mu.rt.p.
rm.mu.rt.d.
rm.f.
n.trch.
rm.na.
rm.opth.trig.
mu.rt.a.
n.opt.
rm.mu.rt.a.
n.oc'mot.
gn.cil.
gn.Gas.
mu.rt.v.
rm.mu.rt.v.
n.abd.
mu.ob.v.
rm.v.
rm.comm.
rm.mu.rt.p.
rm.mu.qd + pyr
rm.opth.trig
2
n.abd
n.cil.trig.
rm.mu.rt.d
n.cil.trig.
n.cil.oc'mot.
n.oc'mot.
rm.n.cil.oc'mot.
gn.cl.
rm.mu.rt.v.
rm.v.

## PLATE 2.

Fig. 3. *A*, *B*, *C*, Transverse sections of oculomotor nerve of fowl at different points along its course, nerve tracts and cells diagrammatic (*A*, most proximal; *C*, most distal). *D*, Transverse section of oculomotor ciliary nerve. Shaded areas indicate regions of small neuraxons; unshaded areas, regions in which large neuraxons predominate. × 33.

Fig. 4. Isolated ganglion cells from ciliary ganglion of fowl. × 233.

Fig. 5. Cells from an embryo in Stage IV (101 hrs. incubation). α, β, Ganglion cells of transitory ganglion of ophthalmic branch of trigeminal nerve. γ, Ganglion cell of mesocephalic ganglion. *cl. comit.*, "Accompanying" cell of ophthalmic branch of trigeminal nerve. Fixed in Zenker's fluid and stained with iron hæmatoxylin. × 933.

Fig. 6. From an embryo *Amblystoma*. *A*, Cells from Gasserian ganglion. *B*, Cells from proximal end of ophthalmic branch of trigeminal nerve. Fixed and stained in vom Rath's fluid. × 600.

Fig. 7. Portion of a section from an embryo in Stage V (118 hrs. incubation), showing neuraxons and fibrils from different points along course of the oculomotor nerve. *A*, proximal; *B*, intermediate; *C*, distal. Fixed and stained in vom Rath's fluid. × 933.

rm.mm.rt.d.
3
C
cl.gn.cil.
A
B
p"n.
D
rm.r.
4
a.
β
γ
5
n'a.r.med.
cl.gn.
cl.comit.
al.
pd.Rac.
cl.comit.
cl.comit.
n'a.r.
A
A
n'a.r.
B
n'a.r.
ε
cl.comit.
cl.comit.
cl.comit.
... n'a.r.
C
B

## PLATE 3.

Fig. 8. Parasagittal section through ventral wall of mid-brain of an embryo in Stage I (72½ hrs. incubation), viewed from the left side. Early neuroblasts of oculomotor nidulus. Fixed and stained in vom Rath's fluid. × 1300.

Fig. 9. Section transverse to longitudinal axis of mid-brain of an embryo in Stage II (70 hrs. incubation), showing root of oculomotor nerve. Fixed in Zenker's fluid, and stained with Brazilin. × 800.

Fig. 10. From an embryo in Stage V (119½ hrs. incubation). $\alpha$, $\beta$, $\gamma$, Ganglion cells in ophthalmic branch of trigeminal nerve; $\alpha$, near Gasserian ganglion; $\beta$, opposite ciliary ganglion in position marked $\beta$ in Figure 25; $\gamma$, more distal, in position marked $\gamma$ in Figure 25. $\delta$, Ganglion cells in ciliary ganglion. Fixed in Zenker's fluid and stained with iron hæmatoxylin. × 1400.

par.ms'c.v.
n'bl.''
cl.comit.
n'ax.
vel.marg.
β
γ
mb.lim.ex.
8
n'bl.'
cl.med.mig.
ms'ench.
vel.marg.
cl.comit.
10
par.ms'c.v.
δ
cl.
cl.'
cl.comit.''
cl.comit.
9
n.oc'mot.
ms'ench.

## PLATE 4.

FIG. 11. Section transverse to longitudinal axis of mid-brain of an embryo in Stage III (88 hrs. incubation), showing root of oculomotor nerve. Fixed in corrosive-acetic mixture and stained with iron hæmatoxylin. × 600.

FIG. 12. Reconstruction from seven consecutive parasagittal sections through an embryo in Stage III (93 hrs. incubation), viewed from left side, and showing longitudinal section through distal portion of ophthalmic branch of trigeminal nerve. Fixed and stained in vom Rath's fluid. × 66.

FIG. 13. Diagram of relations between fundament of ciliary ganglion and ophthalmic branch of trigeminal nerve. Reconstructed from 28 consecutive sections transverse to longitudinal axis of mid-brain of an embryo in Stage IV (100 hrs. incubation). In the Figure dorsal is up, median to the left. Ganglion cells $\alpha$, $\beta$, $\gamma$, $\delta$, $\epsilon$, correspond to ganglion cells with the same designations in Figure 16 (Pl. 5). × 200.

FIG. 14. From an embryo *pig*. *A*, Proximal end of neuraxon of oculomotor nerve. *B*, Longitudinal section through oculomotor nerve midway in its course. Fixed in corrosive-acetic mixture and stained with Brazilin. × 733.

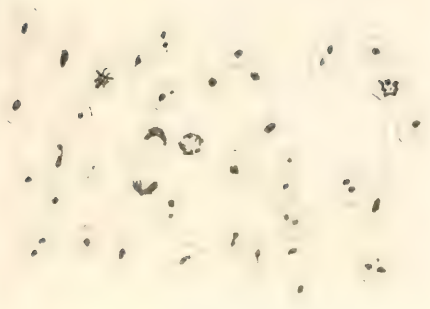
pdl.oc'mot.
cl.
cl.
trt.fbr.v.
ms'cnch.
n.oc'mot.
11
prose.
v.crd.a.
gn.t'i.
rm.opth.trig.
pdl.opt.
12
mb.lim.ex.
A
rm.opth.trig.
n'a.c.
α
β
14
n.oc'mot.
rm.crd.a.
γ
δ
ε
B
gn.cil.
par.rtn.i.
par.rtn.ex.
n.oc'mot.
13

## PLATE 5.

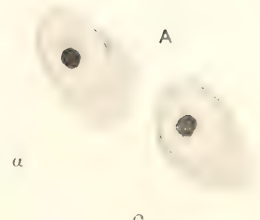
Fig. 15. From an embryo in Stage III (88 hrs. incubation), showing cells of distal enlargement of oculomotor nerve. Fixed in corrosive-acetic mixture and stained with iron hæmatoxylin. × 900.

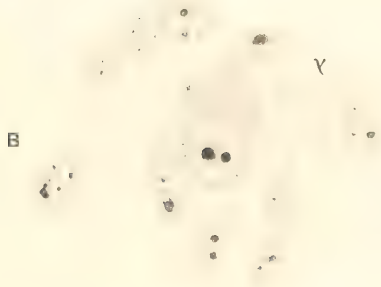
Fig. 16. From an embryo in Stage IV (100 hrs. incubation). *A*, Ganglion cells in ophthalmic branch of trigeminal nerve; *B*, *C*, cells in mesenchyme between ophthalmic branch of trigeminal nerve and fundament of ciliary ganglion (γ, δ, ganglion cells); *D*, ophthalmic-ganglion cells within ciliary ganglion; *E*, ganglion cells of ciliary ganglion (*cl.'*, *cl.''*, "accompanying" cells in ciliary ganglion, which should have been lettered *cl. comit.*). Fixed in corrosive-acetic mixture and stained with iron hæmatoxylin. × 1400.

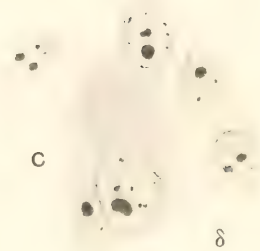
Fig. 17. Longitudinal section through left oculomotor nerve proximal to ciliary ganglion. From an embryo in Stage IV (100 hrs. incubation). Fixed in corrosive-acetic mixture and stained with iron hæmatoxylin. × 900. Note. — The lettering *gn. cil.* should have been *cl. gn.*



15



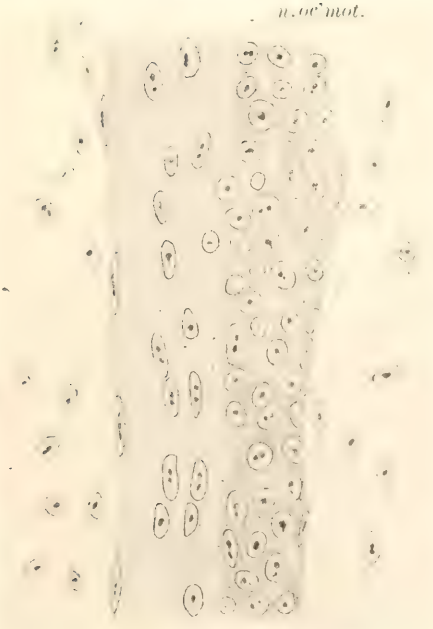




16

17

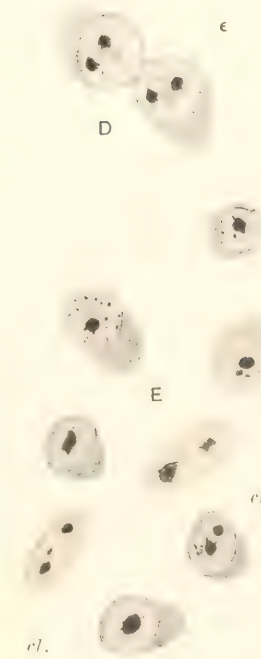
*ms'ench.*

*n.oc'mot.*



*gn.cil.*

*cl.comit.*

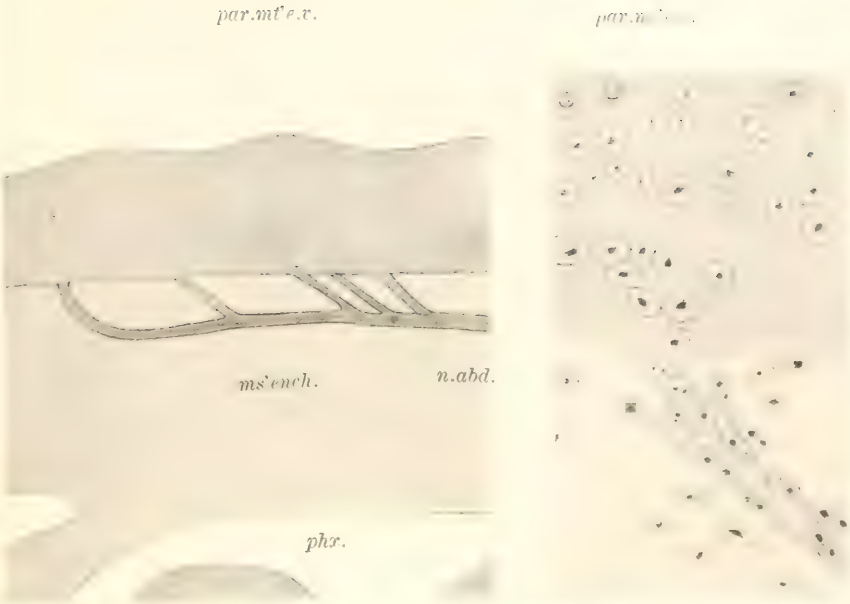


## PLATE 6.

Fig. 18. Reconstruction from five consecutive parasagittal sections through ventral wall of hind-brain of an embryo in Stage V (118½ hrs. incubation), giving a diagrammatic view of proximal end of left abducent nerve, viewed from right side. × 150.

Fig. 19. Enlarged view of a root of the abducent nerve shown in Figure 18. Fixed in picro-sulphuric mixture and stained with Delafield's hæmatoxylin. × 800.

Fig. 20. Parasagittal section through an embryo in Stage III (93 hrs. incubation), viewed from left side, showing root of oculomotor nerve. Fixed and stained in vom Rath's fluid. × 160.


par.mt'e.x.
par.m
ms'ench.
n.abd.
phx.
18
cl.comit.
n.abd.

widl.oc'mod.
trl.fbr.x.
20
ms'ench.
n.oc'mod.

## PLATE 7.

*All the Figures of this Plate are from photomicrographs.*

Fig. 21. Transverse section of oculomotor nerve of fowl corresponding to *A*, Figure 3 (Pl. **2**). × 86.

Fig. 22. Transverse section of branch of oculomotor nerve of fowl supplying dorsal rectus muscle. × 86.

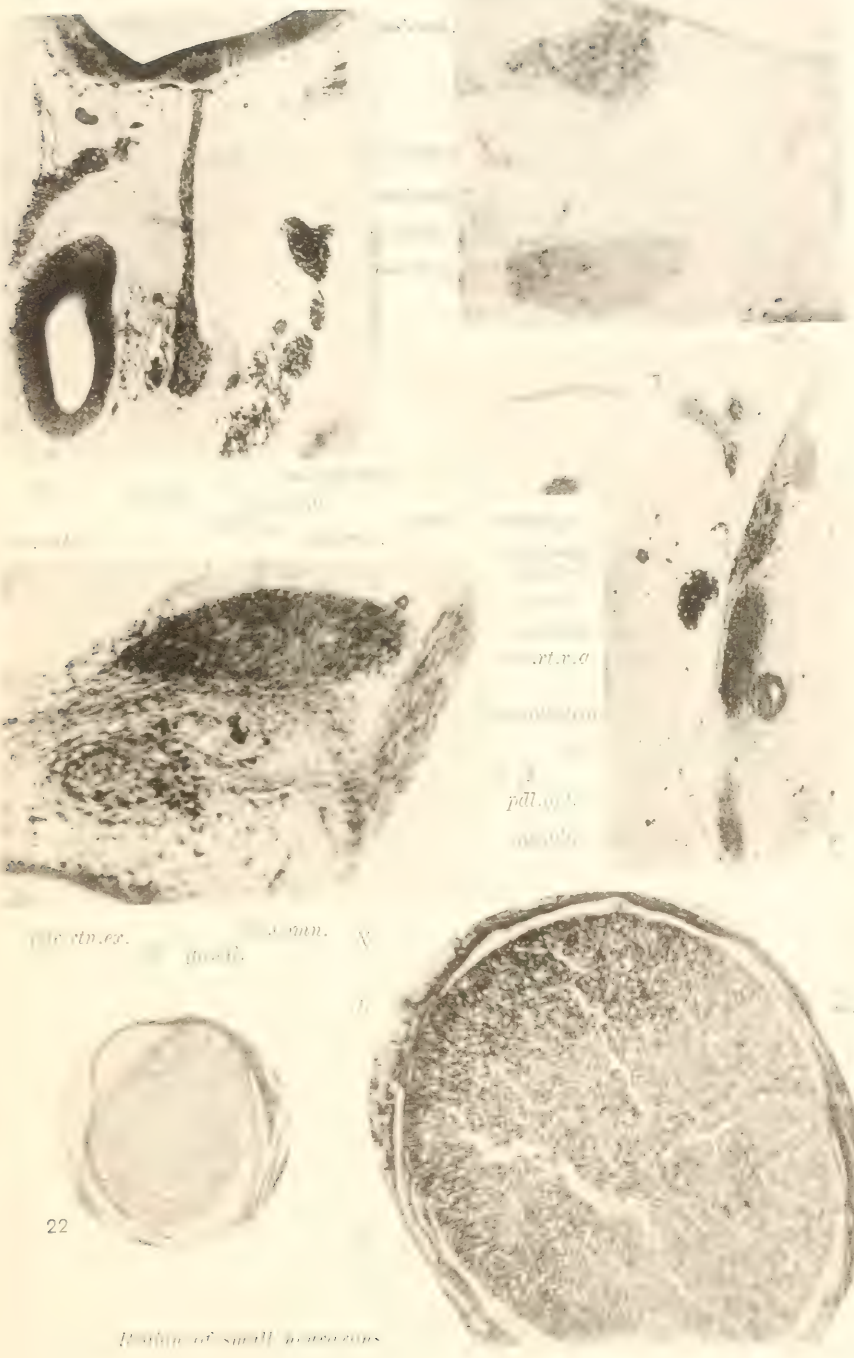
Fig. 23. Section frontal to hind-brain of an embryo in Stage II (70 hrs. incubation), showing connection of maxillo-mandibular portion of Gasserian ganglion with ectoderm, and fundament of posterior rectus muscle. Stained in hæmalum. × 266.

Fig. 24. Section transverse to longitudinal axis of mid-brain of an embryo in Stage III (88 hrs. incubation), showing longitudinal section through entire length of oculomotor nerve. Fixed in corrosive-acetic mixture and stained with iron hæmatoxylin. × 44.

Fig. 25. Parasagittal section of an embryo in Stage V (119½ hrs. incubation), viewed from right side, and showing longitudinal section through communicating ramus between ophthalmic branch of trigeminal nerve and ciliary ganglion. For significance of **β** and **γ**, see explanation of Figure 10 (Pl. **3**). Fixed in Zenker's fluid and stained with iron hæmatoxylin. × 173.

Fig. 26. Parasagittal section through an embryo in Stage V (119½ hrs. incubation), viewed from right side, and showing oculomotor nerve, ciliary ganglion, abducent nerve, and fundaments of eye muscles. Fixed in Zenker's fluid and stained with iron hæmatoxylin. × 40.

Note. — By an oversight the *plus* sign (+) has been omitted from the Plate in the abbreviation *mu. rt. v.* + *a.*


mot.
art.v.a
pdl.sph.
ple.rtn.ex.
mod.
22

No. 3. — CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE, UNDER THE DIRECTION OF E. L. MARK, No. 174.

# *The Eyes of certain Pulmonate Gasteropods, with special Reference to the Neurofibrillae in Limax maximus.*

By Grant Smith.

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## Introduction.

The brilliant work of Apáthy ('97) on neurofibrillae has not only led to a modification of the neuron theory, but has also brought about a renewal of interest in the structure of light-recipient organs and a revolution in our conception of them. In molluscs, as in other invertebrates, the discovery of the general form of the light-recipient structures — which, for convenience, we may call rods — long remained the goal of investigators. A concise statement of the main historical facts up to his own time is given by Hilger ('84). I shall pass by the earlier works and refer briefly to those which usher in a more precise histological treatment of the retinal elements.

The first considerable advance in a knowledge of the retina in gasteropods was obtained from studies on the eyes of Heteropoda. These eyes were pointed to by Leuckart ('54, p. 32) as being peculiarly fitted to serve in the solution of problems concerning the finer structure of the light-percipient apparatus of animals in general. Leuckart himself (pp. 27–34) gave an admirably clear account of the mutual relations of optic nerve, pigment, and retinal elements in Pterotrachea, and described in some detail the form, size, arrangement, and relations of the *rods*. In the following year Gegenbaur ('55, p. 166) described somewhat more precisely the retinal rods of the same animal, saying that they consisted of viscid, homogeneous contents surrounded by a clear and delicate

membrane (Hülle). Ten years later Babuchin ('65) made the promising discovery that the *peripheral* layer of the cylindrical rod of Limax is radially striated, and Hensen ('66, pp. 42–45) proved that the *axial* part of the rod in Pteroceras contains longitudinal fibrils. Babuchin stated clearly, for the first time, that the retina is made up of definite cells and that they have such a differentiation that the appearance of zones is produced; that some of the cells are sensory while others are doubtfully so; and that the optic nerve has an immediate, not a ganglionic, connection with the sensory cells. He showed that certain cells (which he called "central cells") in Helix have cilia-like structures which make up the central zone of the retina; but that in Limax the same zone consists of cylindrical structures with radially striated borders. He was thus the first to picture neurofibrillae, although he did not understand their nature. It is true that he was in error as to the character of the "central cells" and the striated nature of the pigment cells; yet, if he had interpreted the "central cell" with its "capital" as sensory and the surrounding pigment cells as indifferent, he would have been in advance of his time by a generation. See Fig. *A*.

The observations of Babuchin and Hensen in regard to the fibrillar nature of the rods, not having been interpreted physiologically, were neglected. The dominating influence of Schultze's ('69) interpretation of the rod in cephalopods and heteropods contributed to this result. Hence, when Hilger ('84) described the mantle of the rod of Helix as cuticular, he merely accepted the prevalent belief that the rod is a substance secreted by the retinal cells. He found the same idea expressed by Greff ('75, '76), who worked on the eyes of the Alciopidae, and by Grenacher ('79), who studied the retina of arthropods. Hilger (compare Fig. *B*.) characterized the mantle of the rods as completely homogeneous and structureless, as cuticular substance secreted jointly by the pigment cells. He thus ascribed to the pigment cells in Helix structural conditions which they do not possess, and although the distal extension of the rod-cell occupies the centre of the rod in all the species he studied, he saw no sign of fibrillae in it.

Simroth ('76) entirely overlooked the rod in the eyes of Helix, Limax, and Pteroceras; and Carrière ('85) omitted the rods in all the gasteropods, even in copying the figures of Fraisse ('81), who also, like most investigators of that period, looked upon the rod-zone as cuticular. Bütschli ('84) agreed with Hilger in all essential particulars and applied the same criteria to the retinas of other groups of animals.

Patten ('86) protested that the light-recipient surface in arthropods

and molluscs could not be cuticular, and gave the first significant interpretation to the fibrillations of the retinal elements. Although he still held, in a vague way, to the cuticular conception of the rod-zone, he initiated the interpretation which regards as untenable the idea, that the recipient organs of the retina can be inert matter; in support of his belief he found an astonishing abundance of what he termed neurofibrils in the rod-zone, as well as in the deeper layers of the retina. On the whole, however, little confidence can be placed in Patten's neurofibrils, for the following reasons: (1) he used a method which in itself is not suited to the differentiation of histological details; (2) a maceration which will dissolve cuticula cannot be expected to leave the delicate nerve fibrils intact; and (3) Hesse, who has recently reinvestigated the eye of Haliotis, one of the forms on which Patten worked, does not con-

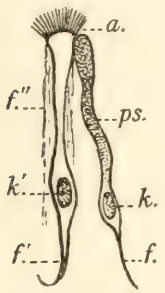


FIG. *A*. Helix pomatia, after Babuchin. *ps.*, Pigment cell, thought to be sensory; *a.*, "Ansatz."

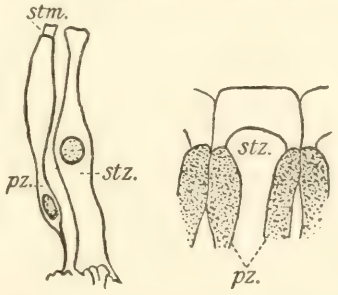


FIG. *B*. Helix pomatia, after Hilger. *stm.*, Rod-mantle; *pz.*, pigment cell; *stz.*, rod-cell; both kinds of cells considered sensory.

firm his observations. Nevertheless, as there is, according to Hesse, a delicate, brush-like rod on the sensory cells of the retina of Haliotis, Patten probably saw some indication of it in his preparations. Patten's studies of neurofibrils preceded those of Apáthy by more than a decade, and he was therefore, consciously, the pioneer in a new field, namely, the study of the finer elements in the peripheral endings of the nervous system. He recognized the significance of the discoveries of Babuchin and Hensen as they themselves did not. He pointed out the futility of considering the presence or absence of pigment a criterion for judging of the sensory character of the retinal cells.

Parker ('**95**) gave the death-blow to Grenacher's theory, that the recipient elements in the eyes of arthropods are cuticular, when he showed that the rhabdomes of the eyes of the crayfish are made up of neurofibrils from the retinular cells. Thus, the erroneous notion that

the light-recipient organs in any group of animals are purely cuticular structures has disappeared and the facts on which such theories were based have been entirely reinterpreted.

Shreiner ('97, p. 45) may be mentioned as one, who, as late as 1897, missed the rods in the eye of Patella.

Henchman ('97), taking up the structure of the sensory cell where Babuchin left it in 1865, made a preliminary report on the eye of Limax maximus, in which she described the histological elements briefly. The unpigmented cells were interpreted as sensory, the pigment cells as indifferent. Thus the correct relations were given for the first time. The axis of the rod as well as the body of the sensory cells was described as "longitudinally fibrous." The fibrillae of the mantle were also recognized. No physiological interpretation of the fibrils was announced. The sensory cells were found to pass into fibres proximally, where, it appears, they were erroneously supposed to connect with the optic nerve indirectly, for an "optic ganglion" is described as located in the base of the nerve. The accessory retina was described for the first time.

Since then Hesse has published a long series of researches on the fibrillae of the rods in many groups of animals and has reinterpreted some of the facts established by former investigators, bringing to his work a painstaking technique and a unity of interpretation which have made his researches notable. His sixth contribution (Hesse, :00), to which reference will be made later, did not deal with the gasteropods. In 1902 (Hesse, :02[a]) he gave a general summary of all his earlier work, and likewise added new researches, which include a study of the retina of gasteropods. Hesse has not used special methods for the differentiation of neurofibrils, and has, therefore, seldom identified them in the cell body; hence, until now, our knowledge of them has been confined mainly to the rod. With the classical work of Apáthy ('97) to point the way, he has shown the significance of the fibrillae of the rods of gasteropods with a clearness which no one before him had reached. He interprets the striated border of the rods, which Babuchin discovered in Helix and Limax, as the recipient, fibrillar endings of the optic apparatus. He believes that, with few exceptions, the essential feature of all light-recipient organs is the presence of neurofibrillae. The axial fibrillae of the rod, such as Hensen found in Pteroceras, he looks upon as intracellular fibrils, which must find their way into the neurite. In the case of one gasteropod, Patella, Hesse (:02[b]) has found a single fibril passing from the brush-like ending in the rod directly through the slender pigmented, sensory cells and thence on into the neurite. Unfortunately,

the retinas of the species in which he has been able to see this fibril best are the least suitable for study, because their sensory cells (as in Patella) are not only exceedingly slender but also pigmented. In the large, unpigmented, sensory cells of Helix and Limax (which are more favorable for study) he has been able to trace the fibrillae only the shortest distance into the cells (see Fig. C). The clearest case which he presents (:00, Fig. 6) is in the eye of an opistho branch, Pleurobranchus, in which he has shown parallel fibrils passing directly through the large sensory cells. Hesse has very convincingly reinterpreted the earlier work of Grenacher (`86) and asserts that the rod of the cephalopod eye contains a single fibril which ends in a little knob distally. He shows that the fibril traverses the length of the cell, being continuous with the neurite. In another article Hesse (:02[a]) describes the rod of Limax more in detail than did Henchman, only the title of whose paper he had seen. In the body of the sensory cells of Helix he (Hesse, :02[b], Fig. 15) represents a striation more or less longitudinal, although he does not call attention to the fact in his text.

FIG. C. Helix pomatia, after Hesse. *nf.*, Neurite of sensory cell; *pzk.*, nucleus of indifferent cell; *p.*, radicula; *st.*, fibrillae.

Thus, knowledge of the fibrillae in the body of the sensory cells of gasteropods, as in other groups, is almost entirely lacking. Furthermore, it has not been established with absolute certainty which of the cells of the retina in pulmonate gasteropods are sensory, for the reason that, hitherto, no one has succeeded in this field with either methylen-blue staining or silver impregnation. In addition, there are certain histological questions of minor importance, the settlement of which would make our understanding of the gasteropod eye more clear.

The present research embraces some of these secondary questions, but is concerned especially with the neurofibrillae of the retina of the pulmonate gasteropods.

I am greatly indebted to Prof. E. L. Mark for supervision and helpful suggestions.

## Material and Methods.

My studies of neurofibrils have been carried out almost entirely on Limax maximus L. For experiments with methylen blue, however, Helix pomatia L. was also used. The aquatic species Planorbis trivolvis Say gave some evidence of pigment-migration.

To secure an eye of Limax or Helix I have usually simply snipped off with scissors the extended tentacle behind the eye and placed the piece in fixing fluid. As complete a penetration of the fixing fluids seems to be secured in this way as by dissecting out the eye from the tentacle, for it is the eye-capsule which offers the most serious difficulty to the penetration of reagents.

The rods were studied in the fresh condition by teasing the retina in 0.9 per cent salt solution. A more dilute solution produces swelling of the rod. In teased preparations, small groups of cells, and especially isolated rods, are abundant. Such preparations were further studied under the polariscope after the method employed by Howard (:**03**). Reference will be made to this method again.

For a maceration fluid one volume of Flemming's weaker mixture was added to nine volumes of water. This solution proved to be satisfactory, for it seems to fix the tissues, and yet they may be torn apart easily. The osmic acid guards against the growth of fungi, which one cannot refrain from suspecting must have found their way into some of Patten's preparations. The eye, having macerated for forty-eight hours or more, was teased into several pieces in a drop of 10 per cent glycerine on a long cover-glass. A square cover-glass with wax feet at the corners was placed over the drop.

By gentle, prolonged tapping on the square cover, the retinal cells were more or less isolated and separated from the capsule. The cells, or groups of cells, could now be studied either from above or below. As the water evaporated from the glycerine, stronger grades of glycerine were added. For convenience the largest possible eyes were chosen for maceration.

I agree with Hesse that Flemming's solution gives the best fixation for ordinary sections. Both acetic-sublimate and Perenyi's fluid were also used. The sections were stained either in brazilin or in Heidenhain's iron-haematoxylin, followed by orange-G. They were usually cut $6\frac{2}{3}\mu$ in thickness, sometimes $3\frac{1}{3}\mu$.

To depigment the molluscan retina without also destroying the retinal cells seems hitherto to have been possible for no one. The method which I have employed is as follows:—

A few crystals of potassium chlorate were placed in a large test-tube containing a little strong nitric acid. A few two-inch glass slides were placed side by side in the bottom of the test-tube. Rising above the acid, these slides not only formed a firm basis which would support any object which might be introduced into the tube, but they also made it impossible

for the objects to come in contact with the acid below. Sections which had been affixed to a slide in the ordinary way were transferred from weak alcohol to water. Then, while the sections were still wet, the slide was lowered into the test-tube until it rested on the upper ends of the two-inch slides, and the tube was at once stoppered with a plug of cotton. By heating the acid a little, chlorine gas was quickly generated, with the result that the pigment was decolored more or less, depending upon the amount of gas and the time during which it was permitted to act. If the gas is allowed to act too long, the cytoplasm is reduced to a structureless mass, but if the action is less prolonged the cytoplasm still resembles to some extent that in sections not so treated. After depigmentation the sections were transferred to water, in which they remained until all of the decolorizer was removed, whereupon they were stained. As it was the purpose of the decoloration to give a view of that part of the sensory cell which passes through the pigment zone, the sections were merely stained in eosin or acid fuchsine. The latter was the more useful stain, because it seems to have the stronger affinity for the cell boundaries.

All attempts to get a methylen-blue impregnation of the retina by injecting the stain into the body were fruitless. But if the eye-bearing tentacles are cut off and placed for an hour or two in a 2 per cent solution of methylen blue in distilled water, the non-pigmented cells of the retina become stained. I employed the ordinary "cold" method, viz., fixing in Bethe's fluid for invertebrates, washing in distilled water or salt solution, passing through grades of alcohol, and transferring to cedar oil or xylol. Sections were afterward made. If the staining is not too prolonged, none of the pigment cells of the retina will be affected. Since the eye-capsule is the last part of the tentacle to be penetrated, the other tissues of the tentacle are heavily overstained. Good preparations of the sensory cells of the surface epithelium, it may be remarked, are also to be obtained by the same method.

For the differentiation of neurofibrillae three methods were used with success. Apáthy's after-gilding method was tried, but, although it is a very good general stain for the retina, I have not been able to make it differentiate the fibrils well. Since Bethe's method employs the same fixation, prolonged effort to succeed with gold chloride was not made.

In using the Bethe method I followed the modification introduced by Prentiss (:03). The tissues were fixed in a saturated aqueous solution of corrosive sublimate for six to twelve hours, and then passed through grades of alcohol up to 70 per cent, in which the sublimate was removed

entirely. Iodine-alcohol was used as a test for the presence of the sublimate, being renewed until it no longer lost color. Tissues treated by Prentiss's method are usually passed through weaker and weaker grades of alcohol to water and then placed in a solution of ammonia (one part ammonia to four parts water) for twenty-four hours, in order to remove any Nissl substance from the sensory cells; but I have not found it necessary to do this in the present case. When the sections were ready for staining, the slides were placed for fifteen minutes or more in an aqueous solution of ammonium molybdate (1:4000), which was kept at a temperature of 35°–40° *C.*, preferably on a water-bath. The slides were then rinsed well in distilled water, finally flooded with it and placed for about a minute in an oven at a temperature of 58° *C.* They were rinsed quickly and stained for not more than five minutes in an aqueous solution of toluidin blue (1:3000) at a temperature of 58° *C.* The excess of stain was rinsed off with distilled water and the slides were passed successively to 96 per cent alcohol, to 100 per cent, and to xylol. The sections should be thoroughly dehydrated and then freed from alcohol, otherwise they are likely to deteriorate rapidly. If the fibrils are not differentiated the sections should be passed through the alcohol more slowly.

Even when the fibrils are properly differentiated, it is often difficult to make out precisely the extent of the sensory cells and to distinguish edges or wrinkles on the surface from neurofibrils. It was therefore instructive to counterstain the sections in a weak alcoholic solution of orange-G.

The fluids devised by vom Rath ('95) are well known. The mixture which I used is described by him on page 282. The tissues were fixed for three or four days in the fluid and then, after being rinsed quickly in methyl alcohol, were placed in crude pyroligneous acid for several days. Dehydrating in the different grades of alcohol was done in the ordinary way, except that the tissues were left in 90 per cent alcohol for several days or until the alcohol was no longer colored. Sections were then made in the ordinary way. Such preparations vary greatly in value. The impregnation with osmium is most complete near the surface; hence the pieces should be made as small as possible. The fluid differentiates the linin substance of the nucleus, but the nuclear fluid is not stained at all. The general fixation seems to be much superior to that obtained by corrosive sublimate or ammonium molybdate. The vom Rath fluid has both the advantage and the disadvantage that it impregnates all of the fibrils in the cell. While, therefore, it reveals the

distribution of the main mass of fibrils, it makes their study difficult; their adequate illustration is impossible.

The *intra vitam* method of Prentiss (:03) rests upon the assumption that, because the methylen blue has a selective affinity for the neuron, it must have a special affinity for just that part of the neuron which has to do with the conduction of impulses. The impregnation was accomplished by the method already described. The fibrillae were differentiated in a 0.9 per cent salt-solution for three or four hours at room temperature, after which they were fixed in ice-cold ammonium molybdate and treated as in the ordinary "cold" method. This method is not successful for the rods, but as these can be so easily studied by simpler methods, the defect is not serious. A very useful addition to the method of Prentiss was made by counterstaining the sections with orange-G; for there is the same need of it here as in the original method of Bethe. The tissues were imbedded in paraffine melting at 60° C., so that the sections wrinkled in cutting very little. After having been attached to the slide with egg-albumen the sections were freed from paraffine in xylol at room temperature. The slides were next transferred to ice-cold xylol for a few minutes, and then to ice-cold 100 per cent alcohol, in which there was dissolved a little orange-G. Thus at the same time water was extracted from the egg-albumen and a counterstain secured. The slides were then returned first to ice-cold, and then to warmer, xylol.

## The Morphology of the Eye of Limax.

The general structure of the eye of snails and its relations to the remainder of the eye-stalk have been described repeatedly. The eye of Limax maximus, however, has never been adequately described, notwithstanding the fact that it departs remarkably from the typical pulmonate eye, and that it is particularly favorable for illustrating the minute structure of this type of eye. I shall therefore begin with a brief account of the morphology of the eye, which will also facilitate an understanding of the sensory cells.

When the eye-stalk is cut off and dropped into a fixing solution, it partly retracts. The eye is thus pulled down into the tentacle for some distance. Flemming ('72, p. 366) found that, in a four per cent solution of potassium bichromate, the eye-stalk will relax in many cases so as to bring the eye back to its original distal position. However, the position which the eye has in the tentacle when fixed is unimportant, since any good fixing reagent will penetrate the tentacle readily. Figure

1 (Plate **1**) is a semi-diagrammatic, longitudinal section of the eye-stalk, which shows the eye retracted. The involution (*ivl. ta.*) in the bottom of which lies the eye, though parallel to the longitudinal axis of the tentacle, is eccentric, being dorsal. The large tentacular nerve (*n. ta.*) runs along the ventral side of the tentacle. In addition to a complicated system of muscle-fibres (*fbr. mus.*), the tentacle contains large masses of unicellular, mucous glands (*cl. gl.*). The remainder of the tentacle consists principally of mesodermal cells supported by abundant connective tissue, among which, here and there, lacunae (*lac.*) are visible.

The retracted eye lies at the proximal end of the invagination. Its distal pole is in contact with the basement membrane of the overlying epithelium, which is here unpigmented and translucent. In form the eye approaches a sphere, but its chief axis is a little shorter than its dorso-ventral axis, for the reason that the so-called accessory retina lies in the antero-ventral part of the eye. The result is that the accessory retina causes a protrusion of the eye in that region and increases the dorso-ventral axis abnormally. The shape of the eye in a longitudinal section at right angles to that of Figure 1 can be seen in Figure 2, which is reproduced from a photo-micrograph of a gold chloride preparation. Figure 18 (Plate **2**), which is similarly reproduced from a vom Rath preparation, shows the eye cut in a plane parallel to that of Figure 1. From a comparison of Figures 1 and 2 it is evident that the invagination (*ivl. ta.*) of the tentacle is wider from side to side than dorso-ventrally. The outline of the eye, as seen in Figure 2, is nearly circular, none of the accessory retina being included. The relative positions of the optic and tentacular nerves in the eye-stalk are shown in Figure 19 (Plate **2**), dorsal being up in the Figure.

An axial dorso-ventral section of the eye reveals the following parts: (1) Optic capsule; (2) Cornea; (3) Retina; (4) Lens; (5) Vitreous humor; (6) Optic nerve; (7) Accessory retina.

The optic capsule (Plate **1**, Fig. 1, *cps. opt.*) is a connective-tissue sheath enveloping the eye. It is very thin distally, but thicker proximally, where it is continuous with the sheath of the optic nerve (*n. opt.*). The capsule furnishes a foundation for the attachment of the cellular elements of the eye.

The cornea and retina together constitute a closed sac, the wall of which is everywhere one cell thick; the sac is attached to the capsule by structures to be described later. As is well known, this cellular sac is derived by invagination from the tentacular epithelium in the course of the development of the animal. The distal third (approximately)

becomes the cornea, whereas the remainder develops into the retina. The cornea (Fig. 2) is an unpigmented layer of cells which are long, slender, prismatic, and translucent. Their arrangement is in general radial, although not strictly so, for their long axes are directed towards the front of the lens, more than half of which lies in the distal hemisphere of the eye. The more proximal marginal cells of the cornea thus either stand at right angles to the chief axis of the eye or their central ends may even point a little toward the distal pole of the eye.

The retina makes up the remaining, or proximal, two-thirds of the optic sac. The peripheral ends of the retinal cells are everywhere in contact with the capsule. The marginal cells of the retina pass in alongside the corneal cells, so that the margins of the two structures are in contact. In general the retinal cells, like the corneal, have a radial arrangement. The position of the pigment makes it convenient to distinguish three concentric zones in the retina, which, however, are somewhat arbitrary divisions, for, as I have stated, the retina is an epithelial structure which is only one cell thick. The peripheral zone (Fig. 1, *rtn. ex.*), in contact with the capsule, is unpigmented. It contains the nucleated portions of the retinal cells. The middle zone (*rtn. m.*) contains the pigment, except for which light from in front would enter the peripheral zone. The internal or central zone (*rtn. i.*) lies between the pigment zone and the lens, and contains the rods, — the light-recipient structures of the retina.

The retina consists of two, and only two, kinds of cells, — pigmented and unpigmented. Both are attached to the capsule at their basal ends, and stand side by side in a radial fashion. They will be described in detail subsequently. The pigment cells are only about two-thirds as long as the unpigmented cells, and they are indifferent; that is, nonsensory. They support the unpigmented cells, which are the sensory elements of the optic apparatus. The pigment cells are the more numerous, particularly toward the margin of the retina, and they are arranged around the sensory cells in such a way as to suggest the appearance of ommatidial groups of cells. That part of the sensory cell which lies distal to the pigment zone is the rod.

Each rod consists of two parts (Plate 1, Fig. 2): (1) a club-shaped axis (*ax. bac.*), which Henchman ('97) rightly interpreted as a longitudinally fibrous, distal prolongation of the sensory cell; in the figure it has the appearance of a dark core; (2) a thick mantle ("Stiftchensaum" of Hesse), which Babuchin ('65) was the first to describe as radially stri-

ated. In the figure it appears as a pale covering (*irlr. bac.*) for the axis.

Proximally each sensory cell gives rise to a neurite, which passes out of the capsule at the proximal pole of the eye to constitute with its fellows the optic nerve. The optic nerve (Fig. 1, *n. opt.*) consists of parallel neurites, and is ensheathed in connective tissue, which is continuous with that of the capsule. Within the capsule of the eye the neurites are arranged in loose strands, which run along the inner face of the capsule among the cells of the retina. The strands converge at the optic nerve and pass out in a manner which will be described later. Pieces of strands may be seen in Figure 18, *n't.* (Plate **2**). Distally the strands grow smaller and smaller toward the cornea, as their neurites are distributed to the sensory cells.

It is not my purpose to describe in detail the lens, which Simroth ('**76**) has treated more fully than any one else. The fresh lens is a spheroidal, highly refractive, apparently homogeneous body, which one can easily dissect out. If alcohol is added to the preparation, the central part of the lens grows porous from loss of water. It then shows the compact, peripheral layer and the porous centre which Simroth discusses at length. Sections of the lens show the conditions more clearly. The lens has no special supporting mechanism, such, for example, as is found in the cephalopod eye, but it may be in contact either with the cornea or with the free ends of the longer rods.

The vitreous humor (Fig. 2, *hu. vit.*) fills the narrow space between the rods and the lens, wherever a space exists, as well as the interstices between the rods. Unfortunately, it becomes brittle in the course of preparation so that it is easily broken into fragments by the knife. In Figure 2 it is less injured than in some cases and its position in the eye is not greatly disturbed.

Except for differences in the size and form of the parts, the facts thus far stated are common to all pulmonates. In Limax, however, there is a remarkable structure at one side of the cornea which was discovered by Henchman ('**97**) and more recently described by Hesse (:**02**). The former refers to it as the secondary or accessory retina, the latter as the Nebenretina. Figure 18, *rtn. acc.* (Plate **2**) shows its location in the antero-ventral part of the eye, at the ventral margin of the cornea. It is peculiar in that it contains no trace of pigment, but consists entirely of sensory cells, interspersed among cells which have the form and appearance of corneal cells. I shall describe the accessory retina in detail later.

## The Histology of the Eyes of Limax maximus and Helix pomatia.

Having described the general relations to each other of the two kinds of retinal cells, I shall now consider each in more detail. Except where it is expressly stated otherwise, the descriptions refer to *Limax*. Inasmuch as the pigment cells are simpler and less important than the sensory cells, they will be considered first.

If the eye be macerated sufficiently, very instructive views may be obtained by preparing the retina between two cover-glasses in the way already described. By this process the retinal cells are more or less completely separated from one another and from the capsule. The pigment cells, isolated and entire, are obtained more easily than the sensory cells by this means. They are elongated and club-shaped with dark-brown granular pigment filling the enlarged distal ends (Fig. 10, compare also Fig. 9, Plate 1). As cross sections show (Figs. 5, 6, 12), the enlarged ends are prismatic, owing to mutual pressure. Near the middle of its length the cell tapers rather abruptly into a slender, cylindrical stalk, whose diameter continues to diminish slowly toward the basal attachment. This stalk-like portion of the cell is clear, unpigmented, and narrower than the nucleus, which consequently produces an enlargement in it. Proximally the slender stalk branches into a number of fine, root-like processes (*rdl'*.), which continue on into the connective-tissue capsule, where they are lost to view in its meshes. It is not unusual in macerated preparations to find two or three separate pigment cells clinging by these roots to the inner face of a piece of the capsule. None of these root-like processes have, as some investigators suppose, the nature of dendrites or neurites. They do not find their way out of the capsule, but end among its fibres. They are, therefore, organs of attachment, whereby the pigment cells maintain their proper place in the retina. The nucleus (*nl'*.) occupies a place near the upper end of the stalk; it is ellipsoidal and nearly twice as long as thick. In macerated preparations it is homogeneous, non-granular, highly refractive and shows no nucleolus.

Tangential sections through the retina at the level of the pigment zone must, of course, show cross sections of a certain number of the retinal cells. Such cross sections have been figured and described repeatedly, but the mutual relations of the cells have never been portrayed as well as they are shown in depigmented sections (Plate 1, Figs. 3, 5–8, 11, 12). If the pigment cells are not too far decolored, they

retain a faint brown tinge, which enables one to distinguish them from the sensory cells, but is not dark enough to obscure the view. Since acid fuchsin has a special affinity for the cell membrane, the outline of the cells stained can be readily distinguished. After such staining it is seen that the pigment cells are grouped around the sensory cells in a fairly definite way. In Figure 5 is shown in cross section a single group of retinal cells. The group contains five prismatic pigment cells, which together surround a smaller polygonal area, the cross section of a sensory cell (*cl. sns.*). In Figure 12 two such groups are shown, and it is to be observed that certain of the pigment cells belong to both groups. There may be as few as four pigment cells in a group (Fig. 11), but I have never seen fewer than four nor more than five which shared in encircling a sensory cell. Henchman, however, says they number from five to seven. The various groups are not separated from each other by narrow spaces such as Hilger ('**85**, Tafel 17, Fig. 19) has shown, but are joined to each other closely, as indicated in Figure 12; the narrow spaces in Hilger's figure are represented by the cell membranes in mine. The cells of the retina are so arranged that the sensory cells are never in contact with each other. Thus, these isolated groups strongly suggest ommatidia, though they have not that regularity of arrangement which is usually characteristic of the latter, nor is the recipient apparatus here a differentiation of that side of the pigment cells which is turned toward the centre of the group, as is probably the case in the ommatidia of most arthropods.

Sections of the retina along the chief axis of the eye when depigmented (Fig. 4, Plate **1**, Fig. 14, Plate **2**) give most instructive views of the relation of the pigmented (*cl. pig.*) to the sensory cells (*cl. sns.*). Three groups of cells are shown in Figure 14. The pigment cells are recognized by their coarsely granular appearance.

There are no neurites arising from the sides or the branches of the pigment cells, as some investigators have declared, and it will be shown later that the pigment cells cannot be light-recipient elements. Hitherto no one has traced out the connections of these indifferent cells except Simroth ('**76**), whose observations contained so many errors that this point seems to have been overlooked by his successors. Inasmuch as the sensory cells in some species (*e. g.* Patella) are pigmented, much confusion has characterized the interpretations placed on the retinal cells of molluscs. Starting from different premises the conclusions of authors have pointed now to the pigmented cells and now to the unpigmented ones as sensory. Reasoning from the structure of the unpigmented cells

in the retina of Limax, Henchman ('97) reached the correct conclusions; but, as Hesse (:02[b]) intimates, only the study of the question by means of *intra vitam* staining or silver impregnation — in the use of which no one has heretofore succeeded — can definitely settle the nature of the cells of the retina.

It is, therefore, a pleasure to have succeeded in the use of methylen blue (see p. 239) on two species of pulmonates, namely, Limax maximus and Helix pomatia. The evidence from this method will be presented in detail in connection with the account of the sensory cells. I desire now only to point out that methylen blue does not stain the pigmented cells of the retinas of these two species, if the staining is checked while the tissues are still alive. Sections of methylen-blue preparations show only the unpigmented cells impregnated, whereas the pigment cells are not only unstained but also in their proximal part so colorless that even their nuclei are usually invisible.

In the *retina of Helix* the pigment cells are essentially like those of Limax, except that their nuclei are a little larger.

The *sensory cell of the retina of Limax* (Plate **1**, Figs. 4, 9; Plate **2**, Fig. 14) has the general form of a long-necked flask. The enlarged end, which contains the nucleus, is proximal and turned toward the capsule; the neck-like portion points toward the lens and pierces the pigment zone, distal to which it is differentiated as the rod.

Typically the proximal end of the cell gives rise to two or more processes. All but one of these processes are radiculae — a term which Grenacher ('86) applied to the special organs of attachment which he found in the retina of a heteropod. Near to the cell body the radiculae are not easily distinguished from the neurite, except in some cases by their direction; but farther away from the cell near the capsule they fail to be stained by methylen blue as intensely as the neurite. At the capsule they branch and pass into its meshes, where they branch again repeatedly like a flat root-system of a plant. I have seen the branches in the capsule only in methylen-blue preparations, in which they appear more or less refractive and faintly blue, or slightly greenish, depending upon whether they are well stained or almost colorless. That these branches are not connective-tissue fibres is evident from their size, which is much greater than that of such fibres; many connective-tissue fibres are impregnated by over staining, and so comparisons are easy. There is no doubt, then, that the sensory cells, as well as the pigment cells, are

provided with special means of attachment. These radiculae are seldom traceable into the capsule, because either they are cut off, as often happens in sections, or, as in methylen-blue preparations, they are not stained well at some distance from the cell. I have been able to follow them best in the accessory retina. A methylen-blue preparation of an accessory retina contained the cell shown in Figure 36 (Plate **3**). This cell gave rise to five radiculae and a neurite. The distal end of the cell points away from the observer, so that the figure shows an oblique, basal view of the cell. One radicula — the one at the left — rises toward the observer as it approaches the ventral wall of the capsule; the others, at different levels, are directed toward the front face of another part of the capsule. Close to the cell the radiculae stain dark blue; farther out they grow faint and indistinct and finally branch at points which are slightly enlarged. Examining in a tangential section the middle layer of the capsule next to the accessory retina, I found many very faintly blue branches which are larger than similar branches from the chief retina, for they arise from larger cells. It was in the study of these tangential sections that I found the small radicula of the cell shown in Figure 28 (Plate **3**). Rising toward the eye, it had escaped notice as I focused sharply on the cell. I have tried to show the diminution of color toward the end of the radicula farthest from the cell by a decrease in the gray tint. Several other figures (Plate **2**, Figs. 14, 17; Plate **3**, Figs. 24, 29, 34) show similar processes. It is possible that none of these processes are neurites, but from the manner of orientation we may reasonably infer that those which point toward the optic nerve are nervous in function. In Figure 34 the cell is viewed from its distal end, the nucleus being seen endwise.

The neurite (Fig. 28, *n't.*) arises from the cell body as a process which at first glance resembles the radiculae, being quite as thick as these. To distinguish this part of the neurite from the more attenuated, proximal part, I shall use the term "neurite-process." At some distance from the cell the diameter of the neurites is much reduced (Figs. 28, 36), so that even with a magnification of eight hundred diameters they appear as fine lines (Figs. 22, 35).

The rod, as I have said, represents the distal part of the sensory cell. Together the rods constitute the internal or central zone of the retina. They are cylindrical or slightly tapering, and rounded at the free end. Each rod is composed of two parts; an axial part, or core, and a peripheral part, or mantle. In my methylen-blue preparations the mantle is always separated from the core by a considerable space, so that the

core appears to lie in a loose sac. But this condition is artificial. I obtained all of my methylen-blue preparations of sensory cells from Limax incidentally, while using methylen blue as a neurofibrillar stain. The less special methods in common use might give better results, but those which I have used are sufficient to serve in identifying the rod as the light-recipient structure, and also to give a needed emphasis to the distinction between the two parts of the rod.

Although ordinary methods of fixing and staining bring out the structure of the rod, only brief mention is here made of its finer structure, since it is to be described in detail in connection with the neurofibrils of the remainder of the cell. It is sufficient to point out here that the essential feature of the mantle is the fine, radially arranged fibrils, which give it a striated appearance (Plate **1**, Fig. 9; Plate **2**, Fig. 14; Plate **3**, Fig. 21). In Figure 14 the mantle on either side of the core appears to rest upon the distal ends of the pigment cells. But the mantle is in no sense a part of the pigment cells, nor does it result from their secretive activity; it is structurally connected to the core of the rod, into which the fibrils of the mantle can be traced.

The core is cylindrical or club-shaped and has its greatest diameter at the base, where it has the form of a funnel, suddenly narrowing into a cylinder, a condition which may be understood more easily by an inspection of Figure 14. The core seems to contain little visible material besides the many parallel fibrils which pass into the body of the cell.

In macerated preparations the rods are found in various states of preservation. The core is always present. Sometimes the mantles are intact, although here, again, they are much separated from the cores, giving the impression that the macerating fluid causes them to swell and push off from the cores. The fluid used was not concentrated enough to plasmolize the rods, neither were the cores shrunken. Often the mantle has been more or less completely torn from the core, a condition which is easily explained in view of the swelling of the mantle. In most cases, if any part of the mantle is present, it is still faintly striated (Plate **1**, Fig. 9). The swollen condition of the mantle is important, because it may help us to understand better the nature of this structure. In these maceration preparations the core shows a conspicuously granular condition. The granules appear to be arranged in longitudinal rows, which, no doubt, are due to the fibrillar structure of the core in the living condition.

Even in depigmented sections, if the decolorizer has not acted too long, the mantle still shows the characteristic radial striations (Fig. 14). The

core of the rod in such a preparation does not show fibrillae, but a granular condition. Thus the mantle is shown to be more resistant than the core. If the decolorizer acts too long, the core appears as a clear, homogeneous, highly refractive substance, which does not stain, while the mantle takes a deep red color. Since the process of depigmentation works destructively on the cytoplasm, the finer details in such sections can, of course, have little significance.

At the level of the base of the rods sections tangential to the retina which have been prepared in the ordinary way occasionally give the impression that the sensory cells are pigmented in that part. That is, one sees the mantle of the rod clearly, but what appears to be the core is entirely obscured by pigment. The matter is easily explained by a glance at Figures 7 and 8 (Plate **1**), which are from depigmented retinas. These figures represent two cross sections of the same cell-group at the distal limit of the pigment. Figure 7 is $6\frac{2}{3}$ $\mu$ proximad to Figure 8, and contains just a little of the red-stained mantle on one side, the left in the Figure. Here are five pigment cells arranged around the rod cell in the typical way. Figure 8, cutting through the extreme tips of the pigment cells, shows the same group surrounded entirely by a red-staining mantle (*ivlr. bac.*), but at two places between pigment cells there are small intercellular spaces which communicate with the vitreous humor. Figure 2 shows that the pigment cells next to some of the rods extend a little farther distad than their neighbors, a fact which gives the distal boundary of the pigment zone an irregular course. Since the mantle extends down over these protruding pigment cells, there is in some sections an apparent pigmentation of the sensory cell.

Although sections $3\frac{1}{3}$ or $6\frac{2}{3}$ micra in thickness show something of the narrow part of the sensory cell which lies in the pigment zone, the presence of the pigment is a decided hindrance to study. With the pigment eliminated by a decoloring method, the sections may be made thicker, with the result that the form of this part of the cell can be seen better. That part of the sensory cell which lies in the pigment zone has a form which is the counterpart of that of the core of the rod (Fig. 14); that is, its funnel-shaped distal end matches the base of the core, and a narrow stem extends through the pigment zone proximally toward the large basal part of the cell. Judging from cross sections through this region (Figs. 3, 5, 6, Plate **1**), the ensheathing pigment cells seem to compress this part of the sensory cell into a prismatic form. There is, however, much variation in the form and size of the sensory cells, especially in the peripheral zone, where they are distorted by the pressure

of other cells into such shapes as Figure 28 (Plate 3) exhibits. The largest sensory cells of the eye (Fig. 28, which lacks the rod) are in the accessory retina. Such a cell may be compared with one from the chief retina of the same eye (Fig. 30) near the cornea.

The relation of each sensory cell to a group has already been mentioned; such a group is shown in side view in Figure 9 (Plate 1). Here a single, long, sensory cell occupies the axis of the group, and is surrounded by four shorter pigment cells. The proximal halves of the pigment cells, being attenuated, do not hide the enlarged, nucleated portion of the sensory cell.

Sections prepared in ordinary ways do not show any fibrillar structure in the vicinity of the nucleus, neither is there in this region any trace of fibrillae in macerated preparations, although Patten ('86) shows most abundant fibrillae in the retina of Haliotis, which he prepared by a maceration process. By depigmenting the sections the cytoplasmic structure is either destroyed or rendered unintelligible, and the nucleus is reduced to a homogeneous ball. In macerated preparations the nuclear structures are probably not accurately preserved. However, the nucleus of the sensory cell may always be identified by its large size, rounded form, fine granulations, and especially by the presence of a large, highly refractive nucleolus. In sections stained with haematoxylin the nuclei of the sensory cells are always seen to be larger than those of the pigment cells, and are very conspicuous on account of their large nucleoli and abundant chromatic substance.

Inside the optic capsule the neurites are gathered into strands, which lie near the inner face of the capsule and converge to the region of the optic nerve, where they pass through several holes in the capsule (Plate 3, Fig. 35), and constitute the optic nerve. In one case nine of these strands were visible in a cross section of the optic nerve at its emergence from the capsule. These strands remain more or less separate from one another for a little way because of the connective tissue surrounding each, and they show a tendency, — first noticed under low powers, — even at some distance from the eye, to group themselves in the form of hollow cylinders (Fig. 22). Figures 22 and 35 are from methylen-blue preparations, and show, in the latter figure, approximately the number of neurites in a strand, in the former, the total number of neurites in the nerve. The neurites, it is seen, are very small compared with the axis-cylinder of vertebrates. The sheath of the nerve, which is continuous with the optic capsule, is about as thick as the latter, except at the point where the nerve arises; here it is thickened. Within the sheath

the connective tissue, except for an occasional nucleus, does not stain. It may be imagined as filling the spaces between the neurites. To impregnate only the neurites, however, it is necessary to stop the staining in time, otherwise the connective-tissue fibrils also will be stained, and thus cause confusion.

Except for the size and shape of the rod, the *sensory cell in the retina of Helix* agrees in every way with that of Limax, as I have described it. Although the proximal part of the sensory cells of Helix stains very successfully with methylen blue (Plate **3**, Figs. 22–24, 26, 27, 29, 33, 34), the rods have not been stained. However, cross sections through the narrow part of the cell and the distal ends of the pigment cells show the sensory cell stained intensely blue. Figure 23 shows three such cells surrounded by pigment granules. The largest cell has a five-pointed outline, because, lying nearer the chief axis of the eye, the cell has been cut at a more distal level than the others, just as it begins to expand into the rounded rod. For a longitudinal view of the rod and sensory cell, see Figure *C* (p. 237), which is copied from Hesse. The other two cells in Figure 23 agree with what was seen in Limax (Fig. 5), except that the cell membranes are not visible.

Since it will be necessary to refer repeatedly to the *accessory retina*, and since it has not been adequately described by other writers, I desire to recur to it again. The relation of the accessory retina to the cornea is seen in Figure 13 (Plate **2**), which shows a section made at right angles to the chief axis of the eye and very near the proximal margin of the cornea. The lens hides the centre of the cornea (*crn.*), whose long, clear cells — polygonal in cross section — are arranged around it in a nearly radial fashion. The small, ellipsoidal nuclei of the corneal cells occupy the basal ends of the cells and thus lie near the capsule. This section of the accessory retina more nearly reproduces all the parts of a complete eye than any other example which I have seen. There are parts of fifteen sensory cells in this one section. According to Hesse the number of these varies from ten to fifteen. Besides the indifferent cells, which, however, have no pigment, there is also a small amount of highly refractive substance (*lns'.*) which has the same consistency as the chief lens, and, in fact, is accompanied by a small amount of vitreous substance. Thus this retina, except that it does not have an optic capsule of its own nor a separate nerve nor pigmented cells, might be called an accessory eye. I have not positively determined whether or not the

accessory retina is separated from the chief retina by a partition of connective tissue, but I think it is not.

The sensory cells of the accessory retina are in all essentials like those of the chief retina, and their impregnation with methylen blue shows that they are probably a functional part of the eye. Two such cells (Plate 3, Figs. 28, 36) have already been mentioned. They are provided with large but typical radiculae, and their neurites accompany to the optic nerve those of the retinal cells of the chief retina in their vicinity. The "neck" portion of the sensory cells of the accessory retina do not suffer so much lateral compression as do the cells in the chief retina, because apparently they are not arranged into ommatidial groups as in the chief retina. Figure 25 shows a part of a very large sensory cell, situated at the front of the accessory retina. Several pieces of neurite are also shown. Figure 36 is a composite drawing made by superimposing the camera sketch from the section which contained the most of the neurite over the one from the next section, which contained the cell body. The two sketches fitted so exactly that there is no doubt as to our having to do here with a sensory cell which shows a relatively long stretch of neurite.

The indifferent cells of the accessory retina represent the pigment cells of the retina in one of their functions only; they serve as a mechanical support to the sensory cells. They have throughout their length the same prismatic form that the corneal cells do; furthermore, they resemble these in being filled with a very inconspicuous cytoplasm. Their nuclei, however, are a little larger than those of the corneal cells. Their basal ends are not refractive and attenuated as are the basal ends of the pigmented cells.

The cells of the accessory retina do not have the radial arrangement characteristic of the chief retina. In relation to the indifferent cells the sensory cells appear to be oriented in a most irregular manner (Plate 2, Fig. 13). Figure 16 shows their haphazard arrangement. It will be seen, for instance, that the two oblique sections of rods in this figure are surrounded by indifferent cells, and that at the same level several other cells are cut through their nucleated part.

There is great variation in this structure, but I have not found an eye of Limax without the accessory retina. In most cases there is no lens; occasionally there is no vitreous humor. When the lens is present it usually has about the same position as in Figure 13 (*lns'.*). In one case it was found in the most ventral position possible in the accessory eye, in contact with the capsule.

Before closing this general account of the structure of the eye, I desire to refer to an *abnormality* which was twice found *in the eye of Limax*. In the first case the preparation was made by the vom Rath method; in the second, the stain was methylen blue. In each there was, near the optic nerve, a secondary outfolding of the optic capsule in such a way that a little chamber was cut off from the main capsule. Connecting the two there was a narrow passage. In the chamber were found the nucleated portions of several sensory cells, their distal parts protruding through the opening into the pigment. Thus the rods of these cells occupied their normal places in the internal or central zone. In one of the cases there was an irregular mass of pigment in the chamber. No effort was made to trace the nerve-fibres, nor to learn how common this abnormality is.

## Some Observations on the Eye of Planorbis.

The eyes of Planorbis differ somewhat from those of Helix and Limax. The shape of the eye has been illustrated by Willem (**'92**[a]). The lens has the general form of a cone, the base being turned outward. The retina conforms to the shape of the lens. The sensory cells, as seen in ordinary sections (compare Plate **2**, Fig. 20, which is a semi-diagrammatic sketch of a part of the retina of Planorbis), appear to resemble those of Limax in every way, except that the rods are shorter, approaching those of Helix in form. The rods are less easily made out than in Limax or Helix, for their fibrillae are more delicate.

The pigment cells vary much more in form than the sensory cells. They may be roughly divided into two groups. To one group belong pigment cells whose nuclei lie in a proximal position next to the capsule; to the other, those whose nuclei lie either in the vicinity of the nuclei of the sensory cells or even distal to them. Both are attenuated proximad of their nuclei, and the latter have the form of long, clear, highly refractive rods. The position of the pigment varies greatly. In general the pigment zone usually appears proportionately much narrower than in Helix or Limax. In the pigment cells whose nuclei lie near the capsule, the pigment may either extend quite down to the nuclei or be confined to a relatively short stretch of the cell, or its proximal limit may reach some point intermediate between these extremes. The same statement applies to the pigment cells whose nuclei lie more distally. In the latter there is usually an enlargement of the cell immediately distal to the nucleus, and this enlargement may be filled with pigment.

When this part of the cell is free from pigment, it appears to contain a vacuole. More distally these cells have a cylindrical form, which they preserve up to the place where they are lost to view in the dense pigment zone. The cells whose nuclei lie near the capsule are very long and slim distal to their nuclei, and do not seem to have the enlargements just described for the other kind. If the pigment extends quite to the nucleus, this part of the cell appears as a thin, black, granular line. The possible width of the pigment zone is thus relatively greater than in Limax; but as the pigment does not ordinarily fill the cells to the nuclei, the zone appears relatively narrower than in Limax.

In spite of the fact that the rods, lying, as they do, distal to the pigment zone, cannot be protected by pigment migration, as can the rods in cephalopods, the variable position of the proximal region of the pigment suggests the possibility of pigment migration in the eye of Planorbis. I therefore attempted to determine by a few experiments whether differing light conditions would produce corresponding changes in the position of the pigment. Several specimens of Planorbis were placed for an hour or more in water in a white, porcelain dish which was set in a sunny window. Their heads were then cut off with scissors and fixed in Perenyi's fluid. Sections were made in the ordinary way and stained in Heidenhain's iron-haematoxylin, followed by orange-G. Similar preparations were made from specimens which had been kept in darkness for an hour or more before killing. Comparisons were then made between the two kinds of preparations in order to learn whether the position of the pigment was different in the two cases. More cases in the "light" eyes had the pigment reaching quite to the nuclei than in the "dark" eyes; but there was such a lack of uniformity in different animals, and even in the same retina, that the evidence was not at all conclusive. Repeated experiments did not lead to more definite results. I am satisfied that the pigment does travel up and down in the pigment cells under the influence of some stimulus, but just what is the exciting factor I have not determined. I have not been able to get any evidence of pigment migration in the eyes of either Helix or Limax.

### Neurofibrillae in the Eye of Limax.

In the following account of the fibrillae I shall first present the evidence concerning them and leave the theoretical considerations, as far as possible, for subsequent discussion. In the figures, beginning with Figure 37 (Plate **4**), darkness of tone or sharpness does not mean that some fibrils in the sections appear darker than others, but that the fibrils

which lie nearer to the eye of the observer have been arbitrarily represented as darker than more remote ones. This remark does not apply to the nuclei.

*Ordinary sections.* Except in the region of the rods, the sensory cells of the retina, when fixed in any ordinary solution (*e. g.* Flemming's) and stained with haematoxylin, do not show fibrillae. In the rods, however, they are very easily demonstrated. Hesse has recently described them for this region and given a text figure of the rod of Limax. He demonstrated that essentially the same relations obtain as in the rod of Helix, whose retina he (:02[b]) has so well illustrated. (See Fig. *C*, p. 237.) Proximal to the rod the fibrils have not been traced hitherto. I shall describe the rods first, coming subsequently to the more proximal parts of the cell.

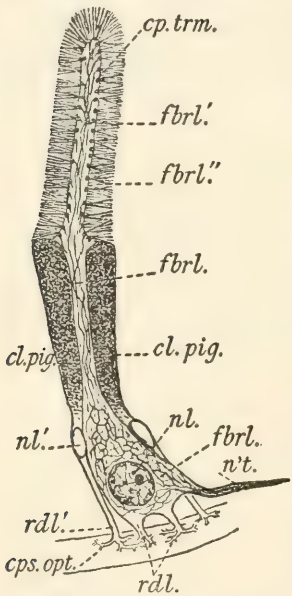


FIG. *D*. Diagrammatic view of a group of retinal cells of Limax maximus. For explanation of abbreviations, see p. 283.

Figure 21 (Plate **3**) shows in cross section five rods. In the spaces between them is the vitreous humor, which envelops the mantle. The rod-axis contains many distinct fibrils. In strictly cross sections, they usually appear at any given focus like so many granules. But if the section is in the least oblique, their fibrillar appearance is unmistakable. Sometimes the fibrils are grouped near the centre of the rod-axis and have a slight twist, so that on focusing they suggest the strands of a loosely twisted rope. Sooner or later the distal part of each fibril turns to the periphery of the rod-axis and ends immediately under the surface of the core in a little knob or knot (Fig. *D*, and Plate **4**, Fig. 49, *cp. trm.*), which Hesse has likened to the basal body in a ciliated cell. For this structure I suggest the name *end-body*, as being a convenient, purely morphological term, without prejudice as to the homology or function of the part. The end-body marks the distal limit of the fibril within the cell; but from the end-body there arises a little tuft of much finer fibrillae, which pass through the surface of the core where they become the fibrillae of the mantle, already described. Unless the fixation is perfect, the mantle fibrils seem to cohere with one another more or less, so that they appear coarser than they really are. This

appearance might be produced by the shrinking together of the mantle in such a way as to bring the fibrils nearer together.

Within the core of the rod the fibrils are approximately parallel and longitudinal. There is no doubt that they are separate from one another — that from their end-bodies to the point where they disappear under the pigment they form neither a network nor a branching system of any kind.

*The fresh rod.* If a large, fresh eye be teased in 0.9 per cent sodic chloride many of the rods remain intact. The mantle now appears like a dim halo around the core, and closer inspection shows that the mantle fibrils resemble fine, motionless, close-set cilia.

I have studied the fibrillar nature of the rod further by means of the polarizing microscope, employing the method which Howard (:03, p. 544) has used recently for the vertebrate rod. "A polarizing microscope was used with a powerful artificial light and a gypsum interference plate inserted between the Nicol prisms. The prisms were placed at such an angle to each other as to give an interference color of a sensitive violet of the first order." Rods, lying parallel to the *a* axis of the gypsum plate, $\pm$ 45° to the cross hairs, were differentiated into two parts. The core of the rod now showed a bright yellow color, whereas the mantle along the side appeared as a dim band filled with blue cross striae. At the distal end of the axis, however, the mantle was yellow like the axis. When the preparation was turned so as to bring the rod into a position at right angles to its former position, the colors were reversed, the core and the distal part of the mantle now becoming blue, and the part of the mantle along the sides of the axis yellow.

These facts (taken in connection with the evidence uniformly obtainable by many methods of fixation) leave no doubt but that the rods of the retina of Limax contain fibrils which are positively anistropic. As far as their optical properties are concerned the fibrils have their axes of maximum elasticity at right angles to their length. The distal end of the mantle gives the same reaction as the core, because the direction of the long axes of the mantle fibrils there coincides with that of the core fibrils. The mantle fibrils on the sides of the core give a reaction opposite to the latter because their long axes stand at right angles to those of the core.

Study of the outer zone of the retina with the polariscope has not given me any definite results, because the elements in this zone are so confused I have not been able to isolate the nucleated parts of the living, sensory cells. The optic nerve reacts like the core of the rods. I shall discuss these results later.

*Fibrillae by the vom Rath method.* Of the four methods of treatment which I have applied to the study of the fibrillae of the eye of Limax, that of vom Rath seems to me to present the best fixation and most faithful representation of the facts. The results differ from those of the Bethe and Prentiss methods in this, that all of the fibrils of the cell are impregnated. From one point of view, this is a drawback, for it makes a study of the individual fibrils difficult. On the other hand, it is good fortune to have some method by which the entire fibrillar contents of the cell may be brought to view. Vom Rath's preparations vary much in value. Sometimes the mantle of the rod appears as a homogeneous, dark-gray covering; but when the impregnation is favorable cross sections of the rod are almost diagrammatic in their distinctness (Plate 4, Fig. 39). The mantle fibrils look like those in an ordinary preparation, except that they less often cohere. The end-bodies appear deep black, and the fibrils of the core very dark and distinct. The matrix between the fibrils of the core, whatever may be its nature, shows no recognizable structure in these sections. Figure 49 is part of a longitudinal section of a rod treated by this method. The mantle appears as a dark-gray cap over the core. Sometimes even in these preparations the mantle is separated from the core. Judging by the size of the cores, it is probable that in some cases the mantle has swollen. It is possible, however, that the core has plasmolized and so separated from the mantle. Figure 46 represents an extreme case. The mantle and core are represented conventionally in a gray tone. The most interesting thing about such a vom Rath preparation is the fact, that from the end-bodies many delicate fibrillae extend across the space between mantle and core. These fibrils are by no means so numerous as those of the mantle, but they give the impression that they are mantle fibrils which were not broken off by the mechanical separation of the mantle and core. It is possible, of course, that these fibrils represent some of the substance either of the end-bodies or of the core fibrils which has been pulled through the periphery of the core.

With the exception of the conditions last described, ordinary sections, such as Hesse has used, show all the details of the fibrils in the rod, which this special method reveals. Inasmuch as the other methods which I have used do not contribute anything further to our knowledge of the rod, the foregoing description must suffice for it.

In vom Rath preparations there is abundant evidence (thanks to the accessory retina) of fibrillae in the narrow middle part of the sensory cells. When the middle part of the cell in the accessory retina is favor-

ably placed, it is easy to observe that the fibrils continue backward from the core of the rod toward the nucleus for some distance practically parallel and separate, just as in the rod. Figure 48 represents one case. The section includes a little of the proximal rim of the mantle, where it has less than a maximum thickness. Imagine the cell so bent that the bit of mantle requires deeper focusing than the other end of the figure. Dozens of dark fibrils issue from the core and run toward the wall of the eye capsule. The fibrils of the mantle are indicated but faintly.

To follow the course of the fibrils through the remainder of the cell is not a simple matter. Their distribution through the cell in most cases is unequal. The largest number usually lie on that side of the nucleus where there is the most room. This fact is sometimes quite noticeable at a magnification of two hundred diameters, even though the fibrils themselves cannot be made out. Where they are most abundant they produce dark spots, one or two, in the cell. This fact is shown by several cells in Figure 16 (Plate **2**). Seen from the side these spots are elongated and might, upon casual observation, be regarded as paths along which sinuous, yet separate fibrils find their way through the cell. Two facts are opposed to such an interpretation. First, careful study of the cells sectioned in all directions leads one to the conclusion that the fibrils form a complicated network in the body of the cell. Secondly, from a comparison of the size of the neurite with the mass of the fibrils in the cell, or even in the axis of the rod, it is clear that the neurite cannot possibly contain as many fibrils as are found in the core of the rod, even if they were so closely packed as to make it practically a solid fibre. There must be a great increase in the cross section of the conducting material in the cell as compared with the cross section of the neurite. Hence there must be either a branching or a network system of some kind within the cell. Granting the difficulties in the way of distinguishing a network from a branching system, it is my opinion, formed after months of study, that in the body of the cell we have to do with a network. Coming from the axis of the rods the fibrils, separate and sinuous, continue down the distal part of the middle portion of the cell for some distance in the same condition. But toward the nucleus the fibrils pass over into a fine and complicated network, which is sometimes diffused throughout the cell, but often is more or less localized. Figure 55 (Plate **4**) illustrates a cell which lay nearly in the plane of the section. The rod (the fibrils of whose mantle are not represented) is bent on itself, so that it is sectioned not quite longitudinally. Immediately proximal to the rod the fibrils (*fibrl.*) still run substantially parallel to

one another, as in Figure 48. Near the nucleus, however, they pass over into a network, whose closeness and intricacy it is impossible to represent adequately. The nucleus seems to be crowded over toward a protruding side of the cell, while the network as a whole passes in a direct course through the cell. There is something of a network all around the nucleus, but except on one side it is comparatively inconspicuous. At any given focus in the network one sees a set of irregularly distributed "knots," which disappear on focusing and give place to a different set. Between the knots run fibrils whose thickness is less than the thickness of the knots. The fibrils at the knots arise in such a way as to preclude the idea that the branching is all distad, the knots being tied together in all directions. There is thus produced the appearance of an irregular network whose meshes vary much in size and shape. The knots do not appear to be the result of accidental fusion or crossing of fibrils, for such relations would require the fibrils arising at the knots to be an even number. This cell is cut off obliquely near the nucleus so that its neurite is not visible.

The network appears likewise in cross sections through the nucleated part of the cell. Figure 51 represents such a section through two cells. In one the network is mainly on opposite sides of the centrally placed nucleus. In the other, it is largely confined to one side of the cell. We do not find here that there are two networks, a circum-nuclear and a peripheral, connected with each other by radial fibrils, such as Apáthy ('97) described for the ganglion cells of the leech.

The neurites which show fibrils are by no means numerous, and there is, therefore, great danger of mistaking radiculae without fibrils for neurites. Figure 54 represents a clear case of a cell whose neurite-process[1] lies nearly parallel to the plane of the section. The most of the neurite is cut away; but the arrangement and direction of the fibrils can be seen. In this cell the main path of the fibrils lies, as was determined by focusing, between the eye of the observer and the nucleus. Immediately proximal to the nucleus there is a series of knots from which fibrils rise upward and take a course as if to pass over the nucleus. Clearly these fibrils were cut away with the section preceding the one shown in the figure. Midway between the nucleus and the neurite there are some good examples of the kind of meshes which the fibrils make. Then, running out into the narrow process of the cell, the fibrils take a more

[1] I have used the term "neurite-process" to indicate that projection of the cell body which merges into the neurite. The fibrils often exhibit a network in this process, but not in the neurite proper.

nearly parallel course. How much of a network they make here it is very difficult to say, for they are much crowded. There are places where there is an appearance of long meshes, but the knots certainly give rise to few fibrils, and they might easily be interpreted as simply the fusions of crossed fibrils. Figure 40 shows another illustration of a neurite-process, but it is cut off before it narrows down into the neurite proper; the cell body, attached to the large end of the process, has not been drawn.

It is very difficult to make out how many fibrils pass down the neurite, either in the accessory retina or in the chief eye. For the neurites run in strands which it is difficult to separate. Moreover, if short bits of single neurites are chosen, it is very difficult to be sure that they are not radiculae. Cells with long stretches of neurite, such as are shown in methylen-blue preparations, are very difficult to pick out in vom Rath preparations. There is a lack of sharp differentiation of parts, which makes the tracing of neurites difficult. Superimposing drawings of successive sections has not yielded any satisfactory results in a study of this matter. Before the use of methylen blue showed me how small the neurites really are, an examination of vom Rath preparations made me believe that I could speak much more confidently of fibrillae in the neurites. I shall return to this aspect of the subject later. From preparations made by the vom Rath method, I have nothing to report as to the neurofibrillae in the strands which within the eye capsule pass toward the optic nerve, for the neurites usually appear like single, fine, dark or black lines. They are very fine as compared with the axis-cylinder of the vertebrate neurite. In the optic nerve vom Rath preparations give some evidence of fibrillae, which I at first thought very good. A longitudinal section of the nerve shows, now and then, more or less nearly parallel, sinuous, black lines. They are probably neurites which have been impregnated more heavily than their neighbors. Parallel with and between them are close-packed, dark-gray lines of about the same tone as the mantle-fibrils of the rod and of the same extreme degree of fineness. I believe that these fine lines represent neurofibrillae. It is possible, however, to interpret them as many, fine, closely-packed semi-transparent neurites whose superimposed outlines give a fibrillar appearance, for, as I have said, the vom Rath fluid, unlike the methylen blue, impregnates all of the neurites.

In the chief retina the fibrillae are visible only in the rods and in that part of the sensory cell which is in the peripheral zone. In the latter they are far more difficult to study than in the accessory retina, because

of the crowding of the elements. Pigment usually obscures the view in the distal part of the sensory cell as far as the nucleus. Proximal to the nucleus (Fig. 41) there is a network, which passes into long meshes toward the capsule, where it merges into the neurite. Finally, the meshes grow so long and narrow that they cannot be distinguished from separate, sinuous fibrils. Tangential sections of the retina fixed in vom Rath's mixture do not yield good results, because it is difficult to distinguish the fibrillae from the pigment-granules.

The nuclei of the sensory cells fixed in vom Rath's fluid have the appearance of large, spheroidal vesicles with a rather scanty network, for the karyoplasm is not stained. The chromatic substance appears in the form of small, dark spherules lodged in the linin substance. There is always a very large single nucleolus.

*Fibrillae by Bethe's Method.* In the sensory cells stained by Bethe's method the fibrils are more or less conspicuous, depending upon how deeply they are stained and how far they are decolored. By overstaining the entire cell is made blue, so that no network is visible; by excessive decoloring the blue of the fibrils, as well as that of the general cytoplasm, disappears. As such sections were also stained with orange-G, the fibrils, when evident, appear against a yellow background. If the blue is all washed out the whole cell appears yellow.

In the rods the fibrils exhibit practically the same arrangement as in vom Rath preparations. There is, however, one difference,—the fibrillae of the mantle are more fused into tufts than when treated by the vom Rath method, because the fixation is inferior. On the other hand, Bethe's method supplements vom Rath's where the latter is almost useless, namely, in the pigment zone of the retina. Cross sections of the sensory cells in the pigment region *distal* to the nuclei exhibit conditions resembling those shown in Figure 23 (Plate **3**). The outlines of the pigment cells are not well defined, but the sensory cells are evident as spots free from pigment, though completely surrounded by it. Under high magnification (Plate **4**, Fig. 42) the cells are seen to contain a close network. Each cross section contains several knots, which are connected to one another and to the periphery by fibrils of smaller diameter. The meshes of the network are finer than in the larger parts of the cell. The network here more than in any other place which I have seen gives the impression of being like a section through alveoli. The knots are relatively so large that they might represent the end views of two or more fused, longitudinal fibrils which run together in the region of the conjunction of three or more alveoli; but such an interpretation is not

borne out by the few longitudinal sections of this part of the cell which I have seen. Moreover, the size of the meshes is visibly greater in Figure 43, even though it represents the second section proximal to that of Figure 42. As in vom Rath preparations, sections still more proximal — those in the region of the nuclei (Fig. 44) — show that the nuclei are more or less eccentric, so that the fibrillar network passes down the cell on one side, — a condition which is maintained even proximal to the nucleus (Fig. 50).

In another respect Bethe's method supplements vom Rath's. Toluidin-blue preparations decolored from all but a few fibrils do not exhibit that confusion of fibrils which results from the use of vom Rath's fluid; thus it is easier to see in toluidin-blue preparations that the fibrils make completely closed meshes and not a distally branching system. These conditions can be observed in a study of some of the cells of the chief retina (Fig. 53), but the cells of the accessory retina offer the more favorable opportunity. Figures 52 and 55 are from the accessory retina, — the former a Bethe preparation, the latter a vom Rath preparation. Comparison of the two will make my statement clear. The latter figure has been described already. In Figure 52 a few loose meshes are to be seen in the neurite process (*prc. n't.*), from which they are traceable distally directly toward the nucleus. Here they unite with meshes on one side of the nucleus. From these meshes fibrils pass toward the periphery of the cell and also distad, probably on their way to the rod. Thus the meshes in the neurite process of this cell correspond, except in number, with those in the process of the cell shown in Figure 55, while the networks in the vicinity of the two nuclei also correspond. The evidence from the Bethe preparations supports the idea of a diffuse network of neurofibrils in the cell body.

*Fibrillae by the Intra-vitam Method of Prentiss.* Doubt as to the reliability of Bethe's method as a specific neurofibrillar stain led me to the use of methylen blue. This method has the advantage over vom Rath's that only a few of the fibrils remain stained, precisely as in Bethe's method. The method brings to view, in properly differentiated cells, a network which repeats all of the essential features described for the vom Rath preparations. Except for the number of fibrils, Figure 37 — which is a section of a cell prepared by the method of Prentiss — might pass for a cell prepared by the vom Rath method; *e. g.*, the one shown in Figure 51. The fibrils are fewer and they are less massed than in the latter figure. The periphery of the cell is too far decolored to show the fibrils distinctly. The larger of the two protrusions incom-

pletely drawn (the one at the left) is the neurite process, which turns abruptly away from the observer. It thus has such a depth and such a complication of fibrils that they cannot be represented. I have tried to work out the network only in the thinner parts of the cell. Close around the deeply-staining nucleus on one side (the upper in the figure) there remains a bluish mass, which, owing to the fact that the excess of stain has not been washed out, hides the fibrils in that region. Having presented already typical sketches of fibrillae by two methods of treatment, I discuss this figure (Fig. 37) simply for comparison. The network has all the appearance of having been a structural condition of the cell previous to the application of the ammonium molybdate. It is hardly credible that accidental fusions could produce such a persistent appearance of net-knots. A casual glance at the figure may suggest the appearance of alveoli, particularly to the left of the nucleus. But with a staining as intense as methylen blue gives, one should be able to see those faces of alveoli which lie parallel to the plane of the section. Such faces, or blue areas, are not present in this preparation, and the trend of the blue lines is for the most part in a longitudinal direction.

The relation of the parallel fibrils in the distal part of the cell to the network in the region of the nucleus is shown by the fortunate bit of selective staining illustrated in Figure 38. The top of the figure is directed toward the rod-zone. At the bottom there are four knots which are tied to one another by short cross-fibrils. From the internal or central knot there branch distally two fibrils; one rises toward the eye of the observer and is cut off, the other extends well out in the direction of the rod. In other directions this knot gives rise to three fibrils which pass to as many knots at different levels laterally. A knot on either side of the first sends fibrils toward the rod. Thus we have clear evidence that the axial fibrils in the core of the rod are in communication with a network in the vicinity of the nucleus. The fibrils are so few and distinct in this example that they could not be confused with the edges of alveoli. The fibrils clearly make a few closed meshes before they give off parallel fibrils to the rod, and the knots are not mere branching places of a distally branching system.

The fibrils in the optic nerve are not so clear. In almost all cases the whole fibre stains so intensely that no hint of a fibrillar condition is obtained. In one of my preparations the staining seems more favorable; but even here the evidence is too scanty. Figure 47 shows a few pieces of selected neurites from the same region as that shown in Figure

35 (Plate 3). One neurite (the left in the figure) seems to be flattened so that its diameter is increased at one region, and throughout this region the neurite seems to be fibrillar. In another example (the central one in the figure) the neurite is cut and seems to be frayed out at the end into several fibrils. In another place (at the right in the figure) there was a larger number of fine, blue fibrils, which had much smaller diameters than the neurites. It is my opinion that each neurite in the optic nerve contains a few fine fibrils.

I have given no examples of the fibrils of the rod stained by methylen blue, for in that part of the sensory cell this stain is very unsatisfactory. Sometimes the fibrils of the core were well differentiated, but in those cases the whole rod was much swollen. Sometimes the mantle-fibrils were sufficiently well impregnated to show that they are nervous substance. Sometimes by a curious selection the end bodies alone are stained.

## General Discussions and Conclusions.

Without entering into controversy as to whether or not the sensory cells of the retina, in the course of phylogenesis, have been derived from the surface epithelium, it is evident from what has been shown in the present research that the retina is an epithelium. Babuchin ('65) was the first to show the single-layer condition of the retina, in regard to which former investigators (Krohn, '37; Leydig, '57, p. 253; Keferstein, '62–66, p. 970, Taf. 83, '64) had left only confusion. Leydig ('65) likewise came to the belief that the various zones of the retina represented only one layer of cells. But it was Hensen ('65) who saw more clearly than any of his contemporaries that the retina is an epithelium one cell thick. At this period, evidence of the epithelial nature of the retina was further increased by many researches in the development of the eyes of molluscs, which cannot be properly reviewed here. There are also several contributions on the adult retina which substantiate the idea that the retina is an epithelial layer. Bergh ('66), whose results I know only through reviews of them by Hilger and others, showed that the cup-like eyes of Fisurella represent simple, incomplete infoldings of the epidermis of the surface of the body. Braun ('79, seen by me only in reviews) and Fraisse ('81) showed that the degree of development of the eye of certain gasteropods (Patella, Haliotis, Fisurella) is an index of the phylo-

genetic position of those genera in the group. The epithelial nature of the retina is again illustrated in the regeneration of the eye, which was studied by Carrière ('80). That the indifferent (pigment) cells of the retina of Limax (to which Hesse has ascribed the function of aiding the corneal cells in the work of secreting the lens and vitreous humor) can have any immediate relationship to the unicellular glands of the external epithelium, as Carrière ('85) supposed, has not been substantiated by evidence. Apparently Carrière's ideas were the natural outcome of his earlier studies on regeneration. He traced the developmental history of the eyes and observed the evident continuity of the retina with the surface epithelium in molluscs having cup-shaped eyes. It therefore seemed logical to homologize the retina directly with the surface epithelium, the unicellular gland cells of the latter corresponding, in his opinion, to the unpigmented cells of the retina, the tactile and other cells, to the pigment cells of the retina. Such a line of reasoning brought him to a conception of the function of the two kinds of retinal cells, which, as the connections of the retinal cells with the optic nerve and the use of methylen blue show, is incorrect. To decide whether the pigment cells really do aid in secreting the lens and vitreous humor will require further investigation.

Emphasis should be placed on the way in which the retinal epithelium is attached to the eye-capsule, which stands in the same relation to the retina as an ordinary basement membrane to its overlying epithelium. The proximal branching processes of the retinal cells, both pigmented and sensory, in Limax and other gasteropods have been described repeatedly and variously interpreted. Babuchin supposed the branches of the pigment cells to be nerve fibres. Being in doubt as to the function of the "central cells" he was silent as to the function of their processes. Hensen regarded the proximal part of the cells as a "fussförmige Abschnitt," while nearer the nucleus a supposed neurite passed off. Simroth was the first to point out that the retinal cells were attached to the capsule, but his observation seems to have been overlooked, possibly because his work contains so much that is erroneous. He described his third type of cell as ending in two or more root-like branches which appeared in the capsule. But similar branches on the sensory cells lead him to suppose some indirect connection with the optic nerve by means of ganglion cells. Hilger, believing that all of the cells of the retina were sensory, supposed that the proximal processes were connected with the optic nerve either directly or through ganglion cells. Grenacher ('86, p. 23) described the special organs of attachment of the sensory

cells in the retina of one of the heteropods (Pterotrachea). He observed that the cell, besides giving off a nerve-fibre, ends in two or more feet, which pass between the bundles of nerve-fibres. Each foot is a great tuft of parallel or branching processes, which pass through into the eye-capsule. Such feet Grenacher called "radiculae," or rootlets, and in his opinion they provide the cells with a special means of attachment. Hesse, finding more than one proximal process on the unpigmented cells of Helix, was reminded of Grenacher's figures, and he very properly applied the same name to them, but did not describe the proximal part of the pigment cells; neither did he demonstrate that the radiculae of the sensory cells passed into the capsule. Both Babuchin and Hilger described the proximal branches of the pigment cells correctly, in my opinion, but their error consisted in failing to trace the branches into the capsule. Simroth's observations remain the only ones which were correct in this matter. In this connection compare the three Figures, *A*, *B* (p. 235), *C* (p. 237), which I have copied from previous writers, with Figure *D* (p. 256), which illustrates diagrammatically the conditions as I have found them in Limax. These radiculae are not "special" organs of attachment, not being different in nature from the similar organs which the pigment cells of the retina and the epithelial cells of the general surface possess. From my figures of methylen-blue preparations, it is evident that the radiculae of the sensory cells of gasteropods are quite different in *appearance* from the structures so named by Grenacher. Although the radiculae branch, they are not in any sense fascicles of parallel branches, but even at some distance from the cell body each is single. Nearer the capsule and, especially within it, there is a copious branching, somewhat like a flat root-system. We see, then, not only that the retina is a single layer of cells, as is the external epithelium of the body, but also that it is attached to a connective-tissue membrane in a similar way. It is not possible to affirm that *all* of the *sensory* cells are so attached, for sometimes the smaller ones are more or less spindle-shaped and do not show radiculae, although such may be present either hidden behind the cell or invisible because unstained.

The sensory cell, although narrow through the pigment zone, expands at the base of the rod to become continuous with its core (Plate **1**, Fig. 4; Plate **2**, Fig. 14). This condition explains the supposed fibre which Babuchin described as occupying an axial position in the cell between the nucleus and the "Ansatz." (See Fig. *A*, p. 235.)

Previous investigators have found it difficult to depigment the retina without entirely destroying the sections. But depigmentation is easily

accomplished to any desired degree, by the method already explained. A less prolonged action of the gas leaves the pigment very faintly brown, so that the pigment cells are readily distinguishable from the sensory cells. Possibly the pigment in the eyes of other molluscs will yield to the same treatment.

Not only does methylen blue as an *intra-vitam* stain give a more accurate picture of the form of the unpigmented cells of the retina than it is possible to acquire by any other means, but, accepting the prevalent view that it selects the nervous cells only, the stain gives conclusive information as to the functions of the two kinds of cells in the retinas of Helix and Limax. The unpigmented cells are undoubtedly sensory, just as Henchman maintained. The failure of the pigment cells to stain helps to corroborate Hesse's view that they probably have no sensory connections. The staining of the sensory cells is so easy by this method that the pulmonate eye offers favorable material for the demonstration of a simple, though clearly unique, type of visual cell. As occasion offers, the same method should be applied to a study of the eyes of those gasteropods in which the sensory cells are apparently pigmented. This method of staining also removes any doubt that might exist as to the functioning of the accessory retina in the eye of Limax.

It is too hazardous to deny that in any pulmonate the indifferent pigment cells can be affected by light. The possibility of pigment migration under the influence of light is not to be overlooked. Parker ('**99**) has shown that in the rhabdome of an amphipod (Gammarus ornatus) the position of the pigment is not the same after the animal has been in the dark for a time as it is in an animal which has remained for some time in the light. In the former case some of the pigment travels in a proximal direction, leaving a part of the rhabdome quite unsurrounded by black pigment. When the same animal passes into the light again, the pigment travels distally to surround the rhabdome. Parker concludes that the pigment in the latter position protects the rhabdome from over-stimulation by light internally reflected from the white pigment. In dim light the withdrawal of pigment permits the internal reflecting apparatus to become effective. I have been able to show, in a research recently published (Smith, :**05**), that the phototropic response of Gammarus annulatus varies under the two conditions. Although the pigment in these amphipods lies in the *sensory* cells, — on account of which the comparison to the gasteropod eye (Planorbis) is not quite exact, — it is probable that pigment movement is a direct response to light-stimulation in Planorbis as well as in the

amphipods.[1] In these arthropods the position of the rhabdome is such that it can be protected by pigment migration, but in the pulmonates the rod is wholly distal to the pigment. Hence, whatever the purpose of the pigment migration, it cannot here be for the same purpose as in the arthropods cited. Some other method of explanation is necessary. However, unless the pigment moves in response to efferent impulses (which is improbable), the reaction is probably a direct response to light stimulation. To that extent the "indifferent" cells may be sensitive. It is true that I have not yet been able to demonstrate a migration of pigment in either Helix or Limax, but in the eye of Planorbis some migration certainly occurs. Not having been able as yet to determine the exact conditions under which it occurs, I can only suppose from analogy that the migration is a response to light. There seems to be less need of pigment migration in snails which have their eyes on retractile tentacles than in those species which, like Planorbis, have their eyes immovably fixed in the surface of the head. The shape of the cells, the position of the pigment in some cells as compared with that in others, and the apparent need of pigment migration in the eye of Planorbis, all point to a probable responsiveness of its pigment cells to light; but the pigment cells are clearly "indifferent" in the sense that they do not transform radiant energy into nerve impulses and transmit them to the central apparatus, for they have no connection with the central nervous apparatus.

Hesse (:02[b], p. 610) ascribes to the pigment of the retina a secondary function, that of insuring "optical isolation." To illustrate with Gammarus, the pigment which surrounds the rhabdome absorbs the oblique rays and prevents their reflection back into the rhabdome. Confusion of images is thus prevented. In dim light the migration of pigment proximad makes it possible for the animal to utilize the reflected light for directive movements. Somewhat similar explanations would hold in the eyes of cephalopods and vertebrates. But in gasteropods the rods are not isolated from each other by pigment, for they are all distal to the pigment cells. No matter how intense the light, the rod is exposed to it, unless the eye be withdrawn into the body, as in Limax, or the head within the shell, as in Planorbis. The pigment zone as a whole absorbs the light which passes through the rods, and thus prevents reflections within the cup-shaped rod zone, and consequently any confusion as to the direction of the source of light. Any other kind of "optical

[1] Somewhat similar pigment migration is known in the eyes of cephalopods and vertebrates, but the mechanism of the movement is not known.

isolation" of the rod is here impossible. This fact may help to explain the small capacity for "vision" which gasteropods show. Although Willem ('92[a]) thinks he has demonstrated that Helix can see — in the ordinary acceptance of the word — an object at a distance of a millimetre, it is evident to one who observes the actions of these animals that vision in the gasteropods is quite subordinated to reflexes of a directive nature, gasteropods being extremely sensitive to differences in light-intensity. The lack of isolation of the rods by pigment explains, in part at least, the lack in this class of molluscs of sharp vision such as the cephalopods possess.

Aside from preventing internal reflections within the rod zone, it is possible that the pigment is directly protective to that part of the visual cell which is surrounded by it. We do not know that the middle part of the sensory cell is not sensitive to light. Neither do we know how or where light-vibrations are transformed into nervous impulse. If the transformation takes place in the middle zone, the pigment may serve some purpose there.

The "optic ganglion" which Henchman ('97, p. 428) mentions as "a funnel-shaped enlargement of the optic nerve containing oval nuclei," is shown by the methylen-blue method to be a misinterpretation, for only connective-tissue nuclei occur there normally. It may be that nucleated portions of a few sensory cells had been pushed out into the base of the optic nerve, as they are in the abnormal protrusions near the optic nerve, already described, and that this condition led to the misinterpretation; or it may be that connective-tissue nuclei were mistaken for the nuclei of the ganglion cells. Thus the old notion of an optic ganglion in the gastropod eye, first introduced by Leydig ('57, p. 253) and adhered to by Simroth ('76), Carrière ('85, p. 16), Hilger ('84), and Henchman ('97) must be abandoned.

In some minor details my observations have not agreed with those of Henchman and Hesse on the accessory retina. Some of the discrepancies may, however, find their explanation in the variability of this structure. Hesse figures the accessory retina as made up of cells arranged exactly as in the chief retina. The sensory cells are given a radial position between indifferent cells, which show a more granular cytoplasm than the corneal cells.

Never, as far as I have observed, is there a regular arrangement of the sensory cells. The typical, irregular arrangement may be seen in Figures 13 and 16 (Plate 2). The indifferent cells of the accessory retina resemble the corneal cells in every way, except that their nuclei are very

slightly larger. The cytoplasmic contents of the two are not conspicuously different either in form or in structure. The indifferent cells are not thin and highly refractive at their proximal ends as are the pigment cells of the chief retina, but their basal ends resemble, instead, those of the cornea. It is therefore too much to say with Henchman ('97, p. 429), respecting the accessory eye: "In one respect only does it differ from the chief eye: the cells corresponding to the pigment cells of the retina contain no pigment. In other respects it presents the same histological conditions and a similar arrangement of histological elements," as in the chief retina. The one constant feature is the presence of the two kinds of cells, but they have such an irregular arrangement and frequently are so bent on themselves (Plate **4**, Fig. 55) that the knife seldom cuts any of them lengthwise in the eyes which I have examined. The indifferent cells of the accessory retina bear little resemblance to the pigment cells of the chief retina, but they do resemble corneal cells, by which they are almost entirely circumscribed laterally. I am, therefore, disposed to believe that the degree of differentiation in the nonsensory cells of the accessory retina puts them into much closer structural relationship to the corneal cells than to the pigment cells of the chief retina.

Hesse gives to the accessory retina a copious vitreous humor, which is continuous with that of the chief retina. The vitreous humor between cornea and lens is made very much more voluminous in his figure than it is in the eyes which I have sectioned. My photomicrographs (Plate **1**, Fig. 2; Plate **2**, Fig. 18) show that there is only a very small amount of vitreous humor in front of the lens. In looking over serial sections of ten eyes, chosen at random, eight showed no connection between the vitreous humor of the two retinas. The two others were doubtful owing to a tearing of the section by the shattered lens. As has already been stated, the accessory retina may have a small lens, though usually there is no accessory lens, and sometimes there is no accessory vitreous humor. In one case the accessory lens was in the most ventral part of the accessory retina, lying against the capsule.

The use of the polariscope in the study of the fresh rods of Limax establishes two facts: (1) The fibrillae are present as a normal mechanism in the living rod; their presence in sections, therefore, cannot be explained on the ground that they may be artifacts produced by the fixing or staining fluids; (2) The fibrils are doubly refractive to light. The rod of Limax adds one more example to those presented by Howard (:**03**), who showed that the light-recipient organs in several groups of

animals are doubly refractive. Howard based his argument for the probable existence of fibrils in the rod of vertebrates partly on the reactions which he observed in the rhabdome of crustaceans, which, as is known (Parker, '95), contains the peripheral endings of the optic apparatus. The rod of Limax furnishes an example which is both more accessible and more to the point, for in the mantle of the rod in Limax the fibrils can actually be seen in the polariscope. Just what is the significance of the double refraction of impulse-conducting fibrils is not yet clear.

The radial mantle-fibrils in the rod of Limax, which Babuchin discovered, and the longitudinal fibrils in the axis of the rod of Pteroceras, which Hensen pointed out, were first described in their proper relations to each other by Henchman. Their function as neurofibrils, which might have been suspected from the interpretations which Patten placed upon fibrils which he observed in the eyes of various molluscs, remained as yet unannounced. From the studies of Hesse we first get a conception of the physiological meaning of fibrils in the rod of the gasteropods. He traced the transition from the single fibrillar brush in the rod of Patella to the broad, fibrillar mantle of Helix, and finally to the large, cylindrical rod of Limax. Such a rod as the last differs in degree, but not in kind, from the single brush of Patella, and Hesse unequivocally ascribed to these end fibrils in the rod of the gasteropods the function of light-reception.

Henchman described both the body of the cell and the axis of the rod as "longitudinally fibrous," and Hesse reported similar indications from the eye of Pleurobranchus. He was able to trace a single fibril through the slim, pigmented rod-cell of Patella into the neurite. In other gasteropods he could only infer the presence of fibrils in the cell body. His figures of the sensory cells of Helix show a very faint longitudinal striation, but he does not refer to the fact in his text. As far as Hesse's observation of the single fibril in the rod-cell of Patella is concerned (or the single fibril in the rod and rod-cell of the cephalopod eye, and even the parallel fibrils in the case of Pleurobranchus), there is no reason to suppose that it is erroneous; for whatever the condition in the pulmonates, it is evident that the fibrils need not be similarly disposed in all groups of molluscs.

There is one thing, however, in ordinary sections of the retinas of Helix and Limax which gives a false impression of parallel neurofibrils in the cell body. It has been shown that, in cross sections through the pigmented zone, the sensory cell exhibits a polygonal outline. Hence in radial sections of the retina one sometimes gets a side view of this

prism-like middle part of the cell. At certain points in the focusing the sharp edges of the prism may come to view in such a way as to be very deceptive. There is great danger of mistaking these sharp edges for parallel neurofibrils. One who has caught a glimpse of the parallel fibrils of the rod as they enter the pigment zone (as in Plate **4**, Fig. 48) might very naturally conclude that the fibrils, although invisible, continue through the cell in the same way as through the rod. Even when the fibrils are brought into evidence, as in the vom Rath method, which gives a confusion of fibrils, the same reasoning would lead to a like conclusion. Influenced by these latter considerations, my preliminary sketches of the sensory cells of the accessory retina showed parallel fibrils passing directly through the cells. It was only after prolonged study of the cells under high magnification that I was forced to conclude that the vom Rath preparations showed a network in the vicinity of the nucleus. This conclusion was subsequently reinforced by the use of the methods of Bethe and Prentiss, which differentiate only a part of the fibrils, thus making the study more easy. The chances of error are so great in the study of fibrillae that special methods should be used wherever possible.

Whatever may be the facts in other gasteropods, all of the evidence from Limax seen in the microscope is in favor of a network of fibrils in the body of the sensory cell. Other considerations show how untenable is the view that the separate fibrils of the core of the rod find their way directly into the neurite. I have shown that the neurites of the optic nerve in Limax are very fine. Even if they contain more than a single fibril, they can scarcely contain as many fibrils as there are in the core of the rod. Large cells with many fibrils, as in the accessory retina, are not represented in the optic nerve by larger neurites than the smaller cells with fewer fibrils, such as are found in many of the cells of the chief retina; but even if they were, it can be shown that the total area of the cross section of all the fibrils in the core of a rod much exceeds the area of a cross section of a neurite in the optic nerve. This last point would hold even if the core fibrils were as fine as the mantle-fibrils. I have already explained why the network cannot represent cross sections of alveoli; and it is most unlikely that fusions of sinuous, though distinct, fibrils would persistently and consistently produce in three different methods of treatment the appearance of a network, even in combination with an alveolar system. It is probable, therefore, that there takes place within the cell a large alteration (increasing distally, decreasing proximally) in the number of fibrils.

There is only one arrangement of fibrils which would so nearly imitate a network in appearance that the possibility of its occurrence in the visual cells of Limax deserves serious consideration. I refer to a distally branching system of fibrils. From the optic nerve the single fibril or the few fibrils of the neurite might enter the cell body and branch dichotomously, or otherwise, in such a way as not to form a network of any kind. Such an arrangement would be difficult to distinguish from a true network, even in perfect fixation, and especially so if accompanied by some fusion of fibrils. If any one insists that there is a branching system in the visual cells of Limax, the idea cannot be well refuted, for we must rely on the microscopic appearances, which might be very deceptive sometimes, as I have just admitted. But the three methods which I have used in this research check one another; and since they present practically the same kind of evidence in each case, it is safe to conclude that there is a network in the vicinity of the nucleus. If we admit that an alveolar condition of the cell is the *embryonic* condition, it is easy to see how a network might arise from an alveolar system by the disappearance of the plane faces and a persistence of the edges of contiguous alveoli. From a physiological point of view it would make little difference whether the fibrils formed a branching system or a network, for in either case the cell would function as a sensory unit.

The most unsatisfactory part of the sensory cell in which to demonstrate fibrils is the neurite. Although on analogical grounds one would expect to find that the neurite consists of at least two or three fibrils, I have been unable to find evidence which would warrant that statement. The neurites of the optic nerve are so small that it is quite possible they may represent single fibrils, as Hesse believes is the case in the neurites from the sensory cells of the eyes of Patella and the cephalopods. But the large number of fibrils in the rod of Limax suggests the likelihood of more than one fibril in the neurite. I have told of the few indications which were found in vom Rath and methylen-blue preparations of a fibrillated neurite, but the evidence is not sufficient to be conclusive.

From the methylen-blue preparations there has been no evidence of a union between sensory cells, such as Hilger imagined. All of the evidence is in favor of the idea that each rod is represented in the optic nerve by a neurite, which is either one fibril or contains fibrils from its own sensory cell only. This arrangement would of course be favorable to sharpness of vision.

The discovery of the accessory retina of Limax was a most fortunate thing for the study of the neurofibrillae of gasteropods, for this structure

furnishes us with a number of very large sensory cells whose details are not obscured by pigment. It is doubtful whether, in the whole animal kingdom, there is a more suitable example for illustrating the fibrillar endings than in the rods of Limax. The accessory retina quickly permits a view of the fibrils in the cell body by both the vom Rath and the Bethe methods. The method of Prentiss is less certain for these reasons: (1) it employs methylen blue, (2) the rods lie within a capsule which is difficult of penetration, and finally, (3) the mantles of the rods seem to offer special difficulties to the differentiation of their fibrils.

The opinions of investigators regarding the nature of the rods have varied greatly. The rod-zone was at first mistaken for the entire retina by Krohn ('37, '39). No one concerned himself with the nature of the rods until Hensen pointed out the axial fibrils. The relatively clear anatomical results of Babuchin did not suggest to him any special function for the fibrils. He assigned light-reception to the pigmented cells, which carried no rods. The rods entirely escaped the notice of Carrière and Simroth. Hensen was the first to commit himself on the structure of the mantle, which he thought was a secretion of the pigmented cells. Along with the acceptance of the opinion that the recipient organs in other groups of animals were cuticular structures, the same idea was applied by him to the mantle of the rod of gasteropods. Hilger urged this conception for Limax, but missed the striae which Babuchin had described twenty years earlier, in spite of the improvements in technique which had meanwhile been made. Hilger believed that each pigment cell of the retinal group produced its quota of the mantle of the projecting rod cells. Patten spoke against the conception that the mantle, being the recipient surface, could be inert, and he introduced the idea of neurofibrils. He believed, apparently, that the fibrils were imbedded in a common cuticular mass, of which the lens and vitreous humor formed a part, and he thought the fibrils were best differentiated by dissolving away the cuticula. Such a course is unnecessary for the rod of Limax, because it shows the mantle fibrils in the fresh state. Patten says (p. 618) "the vitreous body, the lens, and the retinidial layer, at the edge of the optic cup merge into each other and by means of a gradual series of changes pass into the cuticula of the hypodermal cells surrounding the optic cup," thus making the vitreous humor, as I have said, a cuticular structure. It does not appear, however, that this is a correct view.

There are several facts which point to the mantle of the rods as consisting of something besides fibrils. Hesse intimated as much, for he

mentioned as existing between the fibrils a fine granular substance, which osmic acid differentiated, and he suggested that it was a cementing substance. I can confirm that observation to this extent, that in the vom Rath preparations the mantle takes a solid, gray appearance, which is quite distinct from that of the vitreous humor and the axis. In some cases the fibrils are obscure.

The swelling of the mantle in a mixture of one part weak Flemming's solution and nine parts water, or in a dilute physiological salt solution, presents another aspect of the case. In the preparation thus macerated the axis remains of the normal diameter and length; but the mantle (still showing fibrillae) is frequently found to be so elongated that the axis of the rod appears to lie in a water-filled cavity a half or a third longer than itself. In such a case we should expect that the mantle fibrils would be free to withdraw and remain with the axis, unless they were held fast in a resistant substance. Since they do not, it seems that they must be imbedded in a matrix which swells up in the presence of a fluid which is more dilute than the normal fluids.

There is still another observation which bears on the question. It has been mentioned in the general account of the eye that the lens may rest directly against the rods. Hence one sometimes finds rods which are distorted from the usual shape by the pressure of the lens against them. Although the pressure is thus considerable, one can observe that the mantle has its normal thickness and the fibrillae are, as usual, at right angles to the surface of the mantle — not bent or pressed flat against the axis, as one would expect them to be if they protruded freely into the vitreous humor. These considerations make it probable that the mantle is not merely a border of protruding fibrillar brushes bathed by the vitreous humor. The fibrils seem to lie in a cap of formed substance, which is chemically distinct from the vitreous humor and arises, not from the cells which secrete the vitreous humor, but from the rod-axis, which is merely a prolongation of the sensory cell itself. In a sense, therefore, there seems to have been a grain of truth in the idea of the older investigators as to the nature of the rod-mantle. But the view that it is cuticular is so inadequate, as compared with the conception of it as a border where recipient fibrillae find free end, that it must be abandoned in favor of the later view. Not by virtue of its secreted matrix is the mantle the light-recipient portion of the rod, but by virtue of the neurofibrillae which terminate in that matrix. The essential feature of the mantle does not seem to be that its fibrils shall end free in the vitreous humor, like the cilia of Protozoa in their surrounding

medium, but that there shall be a multitude of ultimate fibrils oriented and exposed to the light like the needles on a young pine twig. That the orientation of the fibrils is more easily maintained if they are imbedded immovably in a matrix belonging to the sensory cell is quite evident.

## Summary.

(1) *Intra vitam* staining shows (*a*) that the pigment cells of the retina of Helix and Limax are indifferent, and (*b*) that the pigment-free cells are sensory.

(2) The pigment cells are attached to the eye-capsule by fine root-like proximal branches — the radiculae.

(3) Each sensory cell gives off proximally one neurite to the optic nerve and (probably) one or more branched processes which attach the cell to the capsule.

(4) By the use of the polariscope it is shown (*a*) that the fibrillae of the rods are not artifacts but normal structures of the living cell; (*b*) that the fibrils of the rod are doubly refractive to light.

(5) The neurofibrillae from the rod pass into the cell at first parallel and distinct. In the vicinity of the nucleus they pass over into a network, which gives off one or more fibrils to the neurite. The network is not segregated into two regions, peripheral and perinuclear, as Apáthy found in ganglion cells of the leech, but is either uniformly distributed, or massed into main paths through the cell.

(6) The mantle of the rod contains the ultimate, recipient fibrillae of the optic apparatus, imbedded in a matrix of delicate, passive material produced by the sensory cell.

(7) Histological conditions reveal appearances of pigment migration in the pigment cell of the retina of Planorbis trivovis, but the conditions under which the migrations occur have not been determined.

*Postscript.*

By an unaccountable oversight the preliminary paper by R. Bäcker, "Zur Kenntnis der Gastropodenaugen" (Zool. Anz., Bd. 25, p. 548, 21. Juli, 1902), entirely escaped attention until to-day, when it accidentally came to my notice. The final paper by Bäcker has also been published (Arbeiten Zool. Inst. Wien. Bd. 14), as I learn from the cards of the Concilium Bibliographicum (Zürich). Unfortunately this volume is not accessible in Cambridge.

The main conclusions reached by Bäcker in his preliminary account are as follows: The non-pigmented cells of the retina are sensory; the pigment cells are not. Each of the former terminates at its base in a nerve fibre, distally in

a process (Stäbchen). The fibrillae observable in these cells — which, he says, are undoubtedly neurofibrillae in Apáthy's sense — terminate in the Stäbchen, and these terminations are to be regarded as the light-recipient elements of the visual cells. The clear axes of the pigment cells, seen in Haliotis and Helix, and held by Babuchin and by Carrière to be light-recipient elements, have no claim to that distinction; they are the homologues of the sustentative elements — the glia fibres — of the nervous system, and the pigment cells themselves are the sustentative cells (ependyma) of the retina. The lens and vitreous body are the product of the pigment cells.

It will be seen that conclusions arrived at by Dr. Smith in the present paper regarding the nature of the two kinds of cells composing the retina are in substantial agreement with those of Bäcker, and are the more worthy of consideration since they were arrived at quite independently of the earlier author's work.

E. L. Mark.

Cambridge, February 16, 1906.

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## EXPLANATION OF PLATES.

Except where indicated, the figures refer to *Limax maximus*. They were drawn with the aid of the camera lucida. The neurofibrillae were drawn under a Zeiss $\frac{1}{18}$ homog. immersion objective. Most of the sections were $6\frac{2}{3}\,\mu$ in thickness. Unless otherwise stated, the Figures of Plates **1–3** are magnified 800 diameters.

### Abbreviations.

| | |
|---|---|
| *ax. bac.* | axis of rod. |
| *bac.* | rod. |
| *cl. crn.* | corneal cells. |
| *cl. gl.* | gland cell. |
| *cl. pig.* | pigment cell. |
| *cl. sns.* | sensory cell. |
| *cp. trm.* | end-body of rod-fibril. |
| *cps. opt.* | optic capsule. |
| *crn.* | cornea. |
| *d.* | dorsal. |
| *fbrl.* | fibrillae of sensory cell. |
| *fbrl′.* | " " rod-axis. |
| *fbrl″.* | " " rod-mantle. |
| *fbrl‴.* | " " neurite. |
| *fbr. mus.* | muscle fibres. |
| *hu. vit.* | vitreous humor. |
| *ivlr. bac.* | mantle of rod (involucrum). |
| *ivl. ta.* | involution of tentacle. |
| *lac.* | lacuna. |
| *lns.* | lens. |
| *lns′.* | lens of accessory retina. |
| *nl.* | nucleus of sensory cell. |
| *nl′.* | " " pigment cell. |
| *n. opt.* | optic nerve. |
| *n't.* | neurite of sensory cell. |
| *n. ta.* | tentacular nerve. |
| *pig.* | pigment. |
| *prc. n't.* | neurite-process of sensory cell. |
| *rdl.* | radicula of sensory cell. |
| *rdl′.* | " " pigment cell. |
| *rtn. acc.* | accessory retina. |
| *rtn. ex.* | peripheral zone of retina. |
| *rtn. i.* | central zone of retina. |
| *rtn. m.* | middle or pigmented zone of retina. |
| *v.* | ventral. |

## PLATE 1.

Fig. 1. Median dorso-ventral section of the tentacle and eye; diagrammatic × 52.

Note. — By an oversight *d.* (dorsal) and *v.* (ventral) were interchanged in this Figure.

Fig. 2. Photomicrograph of a gold-chloride preparation; longitudinal section in plane at right angles to that of Figure 1. × 190.

Fig. 4. Single, depigmented cell-group. Compare Plate **2**, Figure 14.

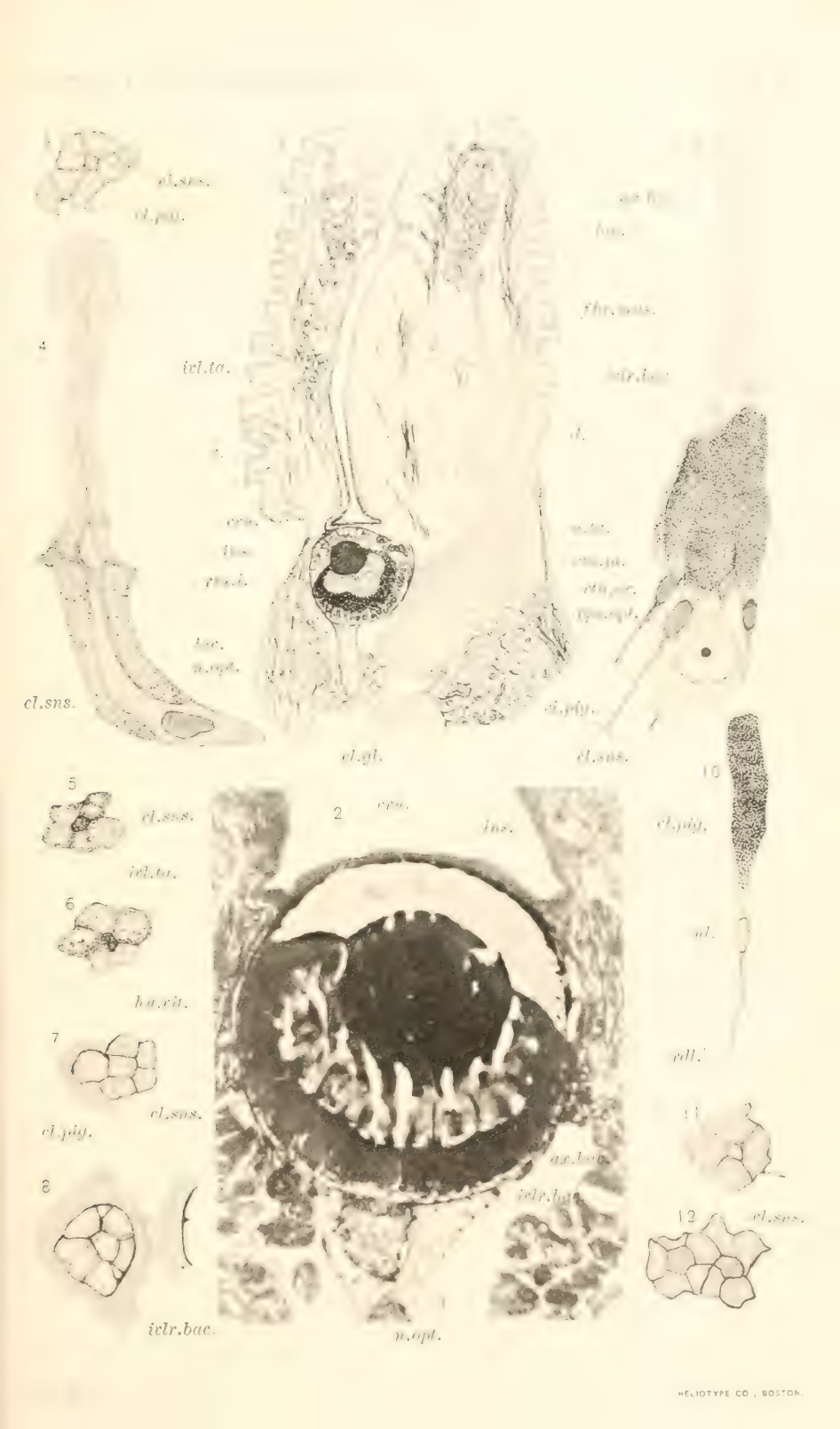
Figs. 3, 5, 6, 11, 12. Cross sections of sensory cell and surrounding pigment cells, successively more proximal; depigmented.

Fig. 7. Similar section through base of rod.

Fig. 8. Similar to Figure 7 and through same cell-group, but $6\frac{2}{3}$ $\mu$ more distal.

Fig. 9. Retinal group from a large macerated eye.

Fig. 10. Single pigment cell from the same preparation as Figure 9.

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## PLATE 2.

Fig. 13. Semi-diagrammatic section of a vom Rath preparation across the chief axis of the eye at the level of the accessory retina. Ventral is toward the bottom of the Figure. × 130.

Fig. 14. Longitudinal section through large cells of a depigmented retina. The sensory cell can be traced through the entire thickness of the retina.

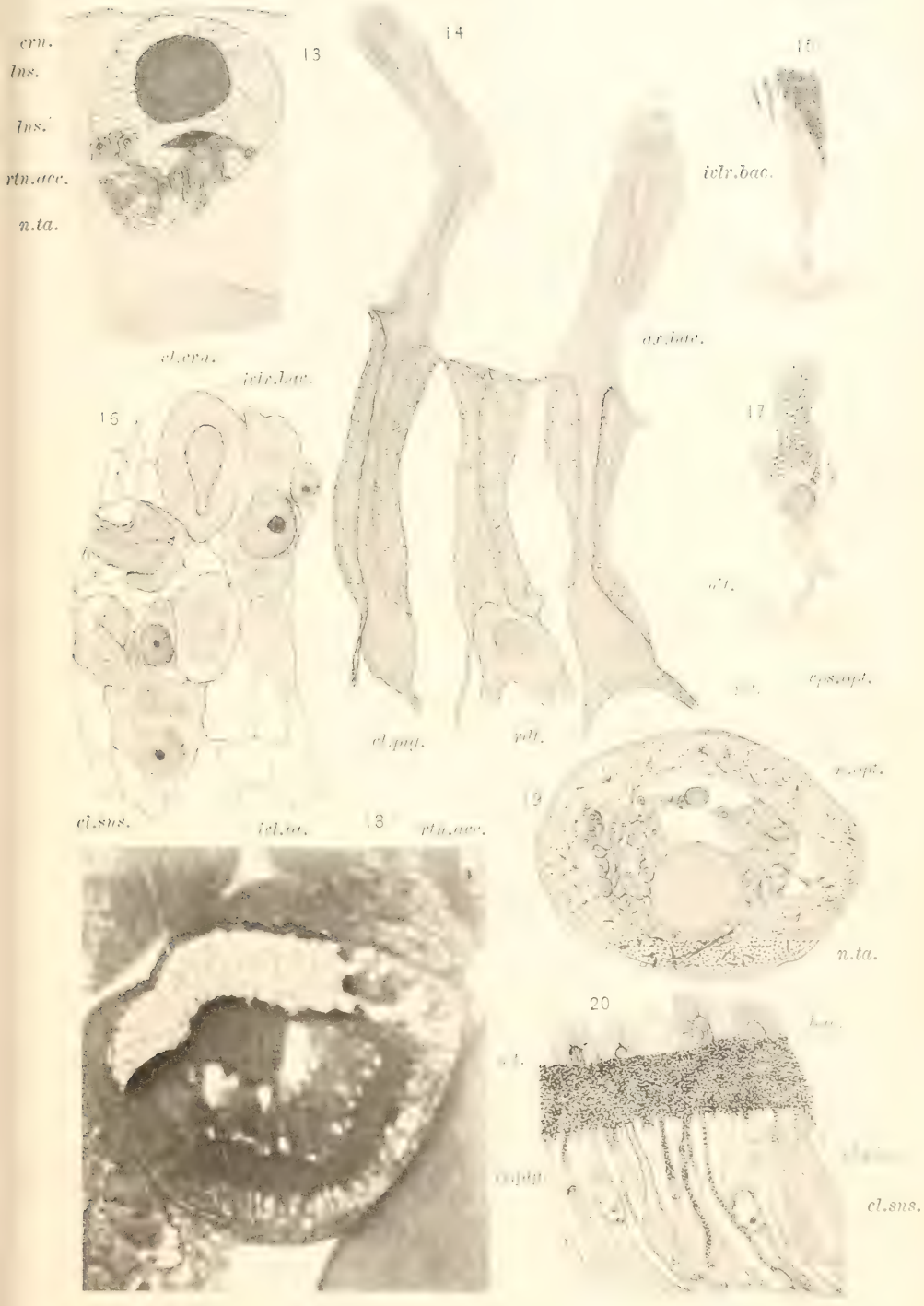
Figs. 15, 17. Methylen-blue preparations of sensory cells.

Fig. 16. Part of section through accessory retina, vom Rath preparation. Several sensory cells cut at the level of their nuclei. Two rods are cut through.

Fig. 18. Photomicrograph of portion of longitudinal, dorso-ventral median section of tentacle, showing location of accessory retina at the right of chief part of eye, i.e. ventral to it. Vom Rath preparation. × 190.

Fig. 19. Semi-diagrammatic cross section of tentacle proximal to the eye, showing the positions of the nerves, the bottom of the figure being ventral. × 52.

Fig. 20. Semi-diagrammatic representation of the retina of *Planorbis trivolvis*, showing the two classes of pigment cells.


crn.
lns.
lns.
rtn.acc.
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icl.r.bac.
cl.exa.
icl.r.lat.
ax.iate.
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a't.
cps.cpl.
cl.pnq.
vdt.
r.cpl.
cl.sns.
icl.ta.
18
rtn.acc.
19
n.ta.
20
l.ac.
a't.
cl.sns.

## PLATE 3.

*Figures 22, 24, 26, 27, 29, 33, and 34 are methylen-blue preparations of* HELIX POMATIA.

FIG. 21. Cross section of several rods.
FIG. 22. Cross section of the optic nerve.
FIG. 23. Cross section of three sensory cells at the distal limit of the pigment zone.
FIG. 24. Sensory cell with radicula and neurite.
FIG. 25. Methylen-blue preparation. One large cell in the accessory retina is sectioned and there are several pieces of neurites.
FIG. 26. Two sensory cells — the distal ends enveloped in pigment — and portion of optic capsule.
FIG. 27. One sensory cell, portions of others and of optic capsule.
FIG. 28. Methylen-blue preparation of a sensory cell of the accessory retina.
FIG. 29. Sensory cell with radicula and neurite.
FIG. 30. Methylen-blue preparation from the chief retina.
FIG. 31. Poorly stained methylen-blue preparation of rod from chief retina. The mantle has separated from the axis or core.
FIG. 32. Methylen-blue preparation, showing three sensory cells and the pale nucleus (*nl'.*) of one pigment cell.
FIG. 33. One sensory cell and nucleus in its relation to the optic capsule.
FIG. 34. End view of a sensory cell cut cross-wise through the nucleus.
FIG. 35. Longitudinal section of the optic nerve where three strands of neurites pass out (downward in the Figure) from the retina through the capsule of the eye. Methylen-blue preparation.
FIG. 36. Basal view of a sensory cell from the accessory retina, showing one neurite and five radiculae. Methylen blue.

21
ax.bas.
22
23
24
cl.sns.
28
29
cl.sns.
n't.
25
26
27
cl.sns.
n't.
cps.opt.
cps.opt.
30
32
33
16

## PLATE 4.

*All figures are magnified 833 diameters.*

Figures 37 and 38 are methylen-blue preparations, made by the method of Prentiss.

Fig. 37. Sensory cell in the accessory retina showing fibrillar network.

Fig. 38. Shows the course of a few fibrils in a sensory cell as they approach the nucleus and form a network.

Fig. 39. Vom Rath preparation, showing the cross section of a rod.

Fig. 40. Longitudinal section of the neurite-process of sensory cell of accessory retina. Vom Rath preparation.

Fig. 41. Sensory cell, chief retina; pigment-cell nucleus. Vom Rath.

Figures 42 to 45 and 50 are cross sections of sensory cells in the chief retina, showing fibrillar network. Bethe preparations.

Figs. 42, 43, 45. Sections through the narrow part of the cell.

Fig. 44. Section through the nucleus.

Fig. 46. Diagrammatic sketch to show the fibrils which stretched across the space between mantle and core in a certain vom Rath preparation.

Fig. 47. A few neurites from Figure 35, which appear to show fibrillae. Such evidence is very rare in methylen-blue preparations.

Fig. 48. Portion of a sensory cell from the chief retina, showing neurofibrils.

Fig. 49. Terminal portion of a longitudinal section of a rod. Vom Rath preparation.

Fig. 50. Section of a sensory cell proximal to its nucleus. Compare Figs. 42–45.

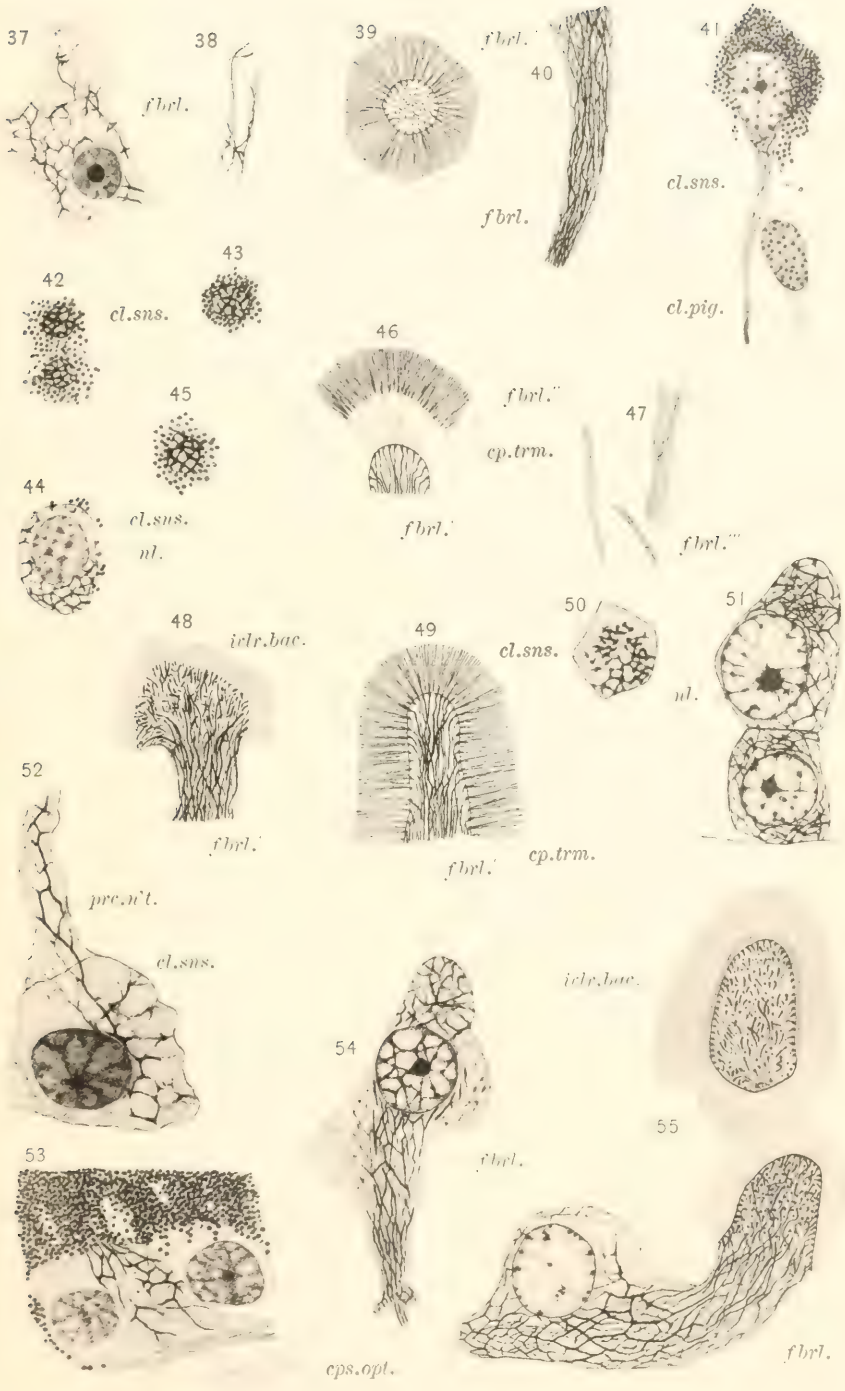
Fig. 51. Cross sections of two sensory cells of the accessory retina cut at the level of the nucleus. Vom Rath preparation.

Fig. 52. Portion of a large sensory cell from the accessory retina. Bethe method.

Fig. 53. A sensory cell from accessory retina bent on itself so that the very base of the rod is seen in cross section and the region (below in the figure) between the rod and the proximal part of the cell is sectioned longitudinally.

Fig. 54. Oblique section of the lower end of a sensory cell from accessory retina, showing fibrillae proximal to the nucleus. Vom Rath preparation.

Fig. 55. Nearly longitudinal section of a sensory cell, showing the way in which the fibrils of the rod finally become transformed into a network in the vicinity of the nucleus.


37
fbrl.
38
39
fbrl.
40
fbrl.
41
cl.sns.
cl.pig.
42
cl.sns.
43
46
fbrl.''
cp.trm.
fbrl.'
47
fbrl.'''
45
cl.sns.
nl.
44
48
iclr.bac.
fbrl.'
49
fbrl.'
cp.trm.
50
cl.sns.
51
nl.
52
prc.n't.
cl.sns.
iclr.bac.
54
fbrl.
55
53
cps.opt.
fbrl.

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AT HARVARD COLLEGE.
VOL. XLVIII. NO. 4.

# STUDIES ON THE NUCLEAR CYCLE OF GONIONEMUS MURBACHII A. G. MAYER.

BY HENRY B. BIGELOW.

WITH EIGHT PLATES.

CAMBRIDGE, MASS., U. S. A.:
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# *Studies on the Nuclear Cycle of Gonionemus murbachii A. G. Mayer.*

By Henry B. Bigelow.

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## I. Introduction.

In the numerous cytologic investigations of the last ten years the sponges and coelenterates have been so neglected that we know to-day very little of the finer structure of their nuclei, either in the "resting" condition or in mitosis. This is the more remarkable when we recall what valuable objects these forms have been in other lines of research, and the interest a knowledge of their nuclear structures must possess on account of their simple organization. In connection with such subjects as spermatogenesis, the individuality of the chromosomes, the disposition of the chromatin in the resting nucleus, and the origin and fate of nucleoli, one might not unreasonably hope to gain an insight into ancestral conditions of importance in the phylogenetic study of the nucleus. These considerations have seemed sufficient reason for undertaking the present research.

The research was originally planned to cover the spermatogenesis of one member of each of the four great groups of coelenterates; but at the outset I was confronted by these obstacles: in most cases the male germ cells are very small, the chromatin in the critical stages exceedingly crowded, and the satisfactory cytologic fixation of the tissues attended with difficulty. Of the various forms examined the hydromedusa Gonionemus murbachii proved most satisfactory in all these respects; it therefore seemed wise to limit the present study to this one species, and to endeavor to follow the entire nuclear cycle from adult somatic cell through the germ cells of both sexes and through fertilization to the cleavage nucleus, and so back to the adult tissue, in the hope of thus laying a firmer foundation for later comparative studies than could otherwise be done. At present I have studied the ordinary mitosis of somatic cells, the cleavage spindles, the entire course of spermatogenesis, the early nuclear development of the oöcyte, and the nuclear

phenomena connected with fertilization. It is my hope in a future communication to consider the subject of the maturation of the egg.

The work has been carried on under the direction of Professor E. L. Mark, to whom I am indebted for unfailing support and helpful criticism; and I also owe a debt of gratitude to the Museum of Comparative Zoölogy for allowing me the use of a laboratory. My thanks are specially due to Dr. Henry F. Perkins, who put at my disposal his collection of the young stages of Gonionemus, as well as to Mr. George M. Gray of Woods Hole, Mass., for his care in preparing the adult specimens of Gonionemus.

## II. Materials and Methods.

The adult specimens of Gonionemus were fixed in Flemming's stronger fluid, which proved entirely satisfactory, giving a beautifully precise fixation, without noticeable distortion or shrinkage of the nuclei. In a few cases excessive blackening occurred with a corresponding difficulty in staining, but this was easily remedied by bleaching, either with hydrogen peroxide or with chlorine, neither of which reagents showed any harmful action. A series of specimens was also prepared with the picric-osmic-acetic-platinic fluid of vom Rath, and, although the cytologic fixation thus obtained was in general inferior, these preparations proved very useful for the study of the achromatic spindle figures and of the centrosomes. For the purpose of studying isolated spermatozoa a dozen or more medusae were prepared according to the isolation method of the Hertwigs (**'78**), the fixation being that of weak osmic acid. For the sake of checking the other methods and for the study of the general structure of the gonad, a number of specimens were preserved simply in 70 per cent alcohol and others in 5 per cent formalin. Finally, I should mention that the material received from Dr. Perkins, on which the study of fertilization and cleavage was carried out, consisted chiefly of entire eggs and of some few series of sections, fixed in part in a corrosive-acetic mixture and in part in strong formalin.

Of a considerable number of staining fluids which were tried by far the most satisfactory for all general purposes was Heidenhain's iron haematoxylin, either alone, or followed by one of the coal-tar plasma stains, orange G, acid fuchsin, Congo red, Bordeaux red, or eosin; while for a check on the iron haematoxylin method, which Boveri (**:00**) has shown to be so essential, Delafield's haematoxylin was employed. For the study of the metamorphosis of the spermatid, iron haematoxylin,

followed by treatment with iodine and then by staining with fuchsin, was very valuable; and for the same purpose the double stain of safranin and gentian violet was of service. As microchemical tests, various mixtures of acid and basic colors were employed, the most useful being the Auerbach mixture of methyl green and acid fuchsin, the Biondi-Heidenhain mixture, and a combination of safranin and Lichtgrün.

The work was at first prosecuted entirely on sections, but I was soon fortunate enough to perfect a method of isolating the cells, which proved of the greatest service. Briefly, it was as follows: a slide was thinly coated with albumen-water fixative, a small fragment of the gonad tissue placed upon it in a drop of 70 per cent alcohol and immediately covered; the cover glass rapidly revolved until the tissue was entirely fragmented, then removed, and the slide plunged into a tube of 95 per cent alcohol. This of course coagulated the albumen, and in the more successful preparations nearly all of the cells were found isolated, 50 per cent, or even more, being entire, at least so far as their nuclei were concerned. The method is made possible by the brittleness of material fixed in osmic acid solutions, and it offers no difficulties, success depending chiefly on the speed of manipulation; while subsequent staining is simple and extremely rapid. The only precaution to be taken is against excessive pressure or prolonged drying, either of which causes distortion of the nuclear contents. In such a study as this, the value of a satisfactory method of isolating the germ cells, especially after fixation, can hardly be overestimated. The advantage of studying the complex chromatin structures on entire nuclei, rather than on sections, — with the accompanying necessity for reconstruction, — is self-evident; the chances of unavoidable error are so much reduced by this device that I feel as though any value this memoir may possess is very largely due to the adoption of this simple means of study. It was used in studying both spermatogenesis and oögenesis; but owing to the difficulty of identifying the cells in the "crushing" method, the examination of the mitoses of somatic cells and of oögonia was made entirely on sections, and these were of course constantly employed as a check upon the other observations.

## III. Historical Survey.

### 1. The Somatic Nucleus.

Although the somatic nucleus was observed in a coelenterate as early as 1872, by F. E. Schultze, it was not until 1879 that Korotneff detected the nucleolus, nuclear network, and granular ground substance. Pfitzner

('83) seems to have been the first to distinguish between chromatin and achromatin in this group; and to reach the conclusion that the nucleus in coelenterates is of the ordinary metazoan type: a conclusion supported by Schneider ('92) and Bidder ('95); and, for sponges, by Fiedler ('88). Sharply opposed, however, to this view, is Chatin ('90), who believes that the nuclei in sponges exhibit decided protozoan affinities.

Hardly more extensive than the literature treating of the resting somatic nucleus in coelenterates is that dealing with somatic mitosis. The earliest study, from a modern standpoint, of this form of nuclear division, is that of Pfitzner ('83), who, evidently stimulated by Flemming's ('79, '80) recent discoveries, examined the process in Hydra. Considering the small size of the cells, and the methods employed, his work is excellent, and deserves more attention than it has received. Moreover, it remained, until very recently, the only detailed account of the indirect division of the somatic cells of a coelenterate. Pfitzner, as already mentioned, distinguishes in the nucleus two substances, chromatin and achromatin; he traces the fate of the former during division, finding that in all important features it conforms to Flemming's ('82) scheme. Five years later Fiedler ('88) described the mitotic figures in sponges; his account is, so far as I have been able to learn, the only detailed one of the process in the adult tissues of this group which has yet appeared. His most important advance over Pfitzner's ('83) earlier work is his ('88, Figs. 30, 31) discovery of the longitudinal splitting of the chromatin rods, and his detection of the centrosome.

The only recent account of somatic mitosis in the adult tissues of a coelenterate with which I am acquainted, is a brief description by Downing (:05) of the process in the interstitial cells of Hydra.

### 2. Spermatogenesis.

The beginning of modern knowledge of spermatogenesis in coelenterates may well be dated from the researches of Eimer ('72), who showed that the spermatozoa were entire metamorphosed cells; not parts of cells or cell derivatives as F. E. Schultze ('71) and Kleinenberg ('72) had supposed. This discovery, moreover, was substantiated by Varenne ('82), who further observed that they arose through repeated nuclear divisions on the part of the sperm mother cells, a result upheld by Polajaeff ('83), Merykowsky ('82), and von Lendenfeld ('83). Thallwitz ('85), who made the first study of the nuclear changes in the spermatogenesis of coelenterates, concludes that, in hydroids at least, the cell divisions pre-

ceding the formation of the spermatozoa are always indirect, and this conclusion is confirmed, for sponges, by Fiedler ('88); but it was not until very recently that Aders (:03) was able to demonstrate that, after the formation of the last generation of spermatogonia, the number of such divisions is invariable, there being formed here, as in other groups, never more nor less than two generations of spermatocytes, and an ultimate generation, the spermatids.

The earliest detailed observations on the metamorphosis of the spermatids are those of Pictet ('91), who describes the process in the case of various siphonophores. He observed the formation of a "Nebenkern" from a condensation of cytoplasmic granules, and the change in chemical composition of the nucleus.

Our present knowledge of the anatomy of the adult spermatozoa of coelenterates is largely due to the researches of E. Ballowitz ('94), who describes them, in medusae and actinians, as being of the ordinary flagellate type. But, in the actinian Tealia, he found an interesting deviation, in that the adult spermatozoa retain much of the appearance of their parent spermatids, thus recalling the conditions described by Pictet ('91) for Halistemma. The observations of Ballowitz have been extended by Retzius (:04, :05), who has described the spermatozoa of many other coelenterates. He has, however, made no contributions to their histogenesis, and it remained for Görich (:03[a], :03[b], :04) to study this process, in Sycandra and Aurelia. Görich's most important observations relate to the rôle of the centrosome in the metamorphosis of the spermatid, and will be fully discussed later.

Of special importance in this connection are the researches of Guenther (:03[a], :04) and Downing (:00, :05) on the nuclear changes in the spermatogenesis of Hydra. Guenther has described a "synapsis" phase in the primary spermatocytes, and has devoted much study to the nucleolus in Hydra viridis, while Downing has given a painstaking account of the entire course of spermatogenesis in the closely allied species H. fusca. To both of these researches I shall have occasion to refer so repeatedly that there is no necessity of summarizing them here.

### 3. The Oöcyte Nucleus.

Kleinenberg ('72), in his classic studies on Hydra, laid the foundations for all more recent investigations of the oöcyte nucleus by the discovery that its ground substance is not homogeneous, as early investigators had supposed, but consists of two distinct materials. This

differentiation was shortly afterwards confirmed by Korotneff ('76) for Lucernaria, and by O. Hertwig ('78[b]) for various medusae and siphonophores. This latter author, who was the first to direct especial attention to the oöcyte nucleolus, found that this structure also was often composed of two different substances, an outer layer and a more transparent central mass.

Most medusae, according to his observation, have only a single nucleolus. But to this rule an exception is found in Eucope, in which, although the young oöcytes have only one, more advanced stages contain several. Nussbaum ('87) and Brauer ('91[a]) have extended the latter observation to Hydra, finding that the germinative vesicle at first contains only one large nucleolus, and that with growth from two to five small ones appear; and Fiedler ('88) has made similar observations on Spongilla.

These studies on the nucleolus are reviewed in detail by Montgomery ('98[b]), who has added a description of the germinative vesicle of a siphonophore, perhaps Rodalia, which contains one large vacuolate nucleolus, and several smaller homogeneous ones, with a different staining reaction. Two different kinds of nucleoli have more recently been described by Conant ('98), and by Morgenstein (:01).

Finally I should mention the studies of Doflein ('96) on the changes which take place in the staining reactions of the various nuclear structures during the growth of the germinative vesicle of Tubularia.

### 4. Maturation, Fertilization, and Early Cleavage.

The papers which relate to maturation, fertilization, and cleavage in coelenterates fall into two groups, according as they do not or do take cognizance of the chromosomes. In the first group are the works of Kleinenberg ('72), Fol ('73), Metschnikoff ('74), Korotneff ('76), O. Hertwig ('78[b]), Claus ('82), Kowalevsky et Marion ('83), Metschnikoff ('86), von Koch ('87), Fiedler ('88), and Hickson ('88). These, in the light of our present knowledge of nuclear phenomena, are now of little more than historic interest. Among them, however, should be especially mentioned, for their importance in the general advance of cytology, Fol's ('73) discovery of the double stellate figure in the egg of Geryonia, and O. Hertwig's ('78[b]) studies on the rôle of the germ nuclei, and on their relation to the cleavage nuclei in Mitrocoma. Earliest in the second group is Boveri's ('90) study on the maturation, fertilization, and early cleavage of the medusa Tiara. In this species Boveri was able to

establish conclusively the occurrence of a numerical reduction of the chromosomes during the maturation of the egg. He showed that the egg nucleus contains only half the number of chromosomes normal to the species, and that after the fusion of the germ nuclei the complete somatic number once more appears in the formation of the first cleavage nucleus. He was, furthermore, the first to count the chromosomes in any coelenterate.

Further details of the process of maturation were soon after presented by Brauer ('91[a]), whose conclusions in the main substantiate for Hydra, the earlier work of Boveri ('90). According to Brauer, the division of the chromosomes in the first maturation mitosis is apparently transverse. To Brauer ('91[b]), furthermore, we owe the discovery that in Tubularia, at least, the sperm nucleus is accompanied during its journey through the egg by a distinct astral radiation.

Boveri's ('90) discovery of the numerical reduction of the chromosomes in Tiara was supported by Häcker ('92[a]), who was able to count six such structures in the second maturation spindle, and twelve in the first cleavage spindle of Aequorea. In this form the nucleolus is cast out of the nucleus before maturation, and long persists in the yolk, — a fact observed also by H. V. Wilson ('94) in the sponges Hircinia and Taedanione. Häcker ('92[a]) further states that the sperm nucleus is accompanied by an aster, and notes the fact, previously detected by Brauer ('91[b]), that while the cleavage spindles have large and prominent asters, the mitotic figures in older larvae show no trace of any such structures.

We owe to Maas ('99) the only detailed description of the maturation, fertilization, and cleavage of a sponge which has yet been written. In Sycandra the chromosomes of the first polar spindle are formed by a condensation of the chromatin microsomes present during the growth period of the egg. The sperm nucleus consists of a chromatic mass, together with a more refractive "middle-piece" which forms the centre of a series of astral radiations. When the two germ nuclei come in contact each contains sixteen chromosomes, which, after fusion, pass without alteration into the first cleavage spindle.

The same stages have been described within recent years in various hydroids, by Morgenstein (:01), Harm (:02), and Wulfert (:02). Among these, Morgenstein's (:01) observations on Cordylophora are of most interest because they contain the first detailed study of the coelenterate centrosome. No centrosome could be detected in either of the maturation spindles, but the sperm nucleus, in its migration

through the egg, is accompanied by a centrosome, which divides into two, forming the centres of two separate asters; and these the author traces directly into the asters of the first cleavage spindle.

Wulfert (: **02**) has reached much the same conclusions in the case of Gonothyrea except that he believes that a centrosome is to be seen within the germinative vesicle before the breaking down of its membrane, in spite of the fact that no such structures are present in the ensuing maturation division. In addition to his observations on the centrosome, Wulfert has followed the details of the union of the germ nuclei, with results to be discussed fully later on. Harm (: **02**), besides confirming the work of the two students last mentioned, was able to trace the history of the chromatin in the maturation divisions of Clava, finding that in the first division there are about sixteen tetrads, while in the second, although the number of chromosomes is the same, each instead of being quadripartite is bipartite.

Finally, two recent papers, one by Hargitt, the other by Hill, deserve mention. Hargitt (: **04**) maintains that in Pennaria there is an apparent dissolution of the germinative vesicle at the time the egg is set free — an apparent phenomenon described by many of the early observers in coelenterates as well as in other groups, but one which has been shown by O. Hertwig ('**78**[b]), Fiedler ('**88**), and others, to be apparent rather than real. Hill (: **05**) goes even further, for he contends that in Alcyonium the polar cells are formed by a direct division of the germinative vesicle, and that there follows a considerable period when the egg is actually, as well as apparently, enucleate. But neither his material nor his figures seem sufficient to establish the actual occurrence of such a remarkable type of development.

## IV. Observations.

### A. Mitosis in the Somatic Cells of Adult Tissues.

In the adult Gonionemus somatic cells are so rarely found in active mitosis that it has taken a long search to find an approximately complete series of the stages. I had hoped to be able to study mitosis on different tissues for the sake of comparison, but because of the rarity of the process I have been unable to do so, and shall therefore limit the description to the endoderm cells which line the radial canals. The few mitotic figures found among entodermic gland cells and ectoderm cells agree in all essentials with those of the endodermal epithelium.

These epithelial cells are of the ordinary undifferentiated type, the

cytoplasm is coarsely alveolar (Figs. 3–11), and frequently encloses spherical masses, probably of metaplasmic nature, which osmosize more strongly than the ordinary protoplasm, are not easily dissolved by either acids or alkalis, and no doubt correspond in their nature to the bodies of similar appearance found in the germ cells of both sexes. There are likewise frequently present in the cytoplasm clusters of the small brown pigment granules which give to the gonads of Gonionemus their characteristic color. No archoplasmic structures of any kind are to be seen except during active mitosis, nor can a centrosome be detected with any certainty, although occasionally a minute granule is to be found, which may perhaps represent that structure. The nucleus, during the "resting" stage, is approximately spherical and measures only 5.5 $\mu$ in diameter, so that it is decidedly smaller than in the spermatogonia (page 302), and agrees closely in size with the nuclei of oögonia and of the ectodermal covering cells of the gonad. As a rule, it encloses a single very large nucleolus, but occasionally two or three are present, all of normal structure, though of rather less than normal size. The nucleolus when stained with iron haematoxylin shows in most cases a dark peripheral ring or shell, enclosing a pale or even transparent central mass (Fig. 3) exactly as is seen in the nucleolus in the spermatogonia (page 302). The deeply staining shell, as we shall see from its later history, is probably composed of chromatin, while the central sphere is to be regarded as corresponding in nature to a plasmosome. This type of compound nucleolus is characteristic of all the adult somatic cells of Gonionemus thus far examined, as well as of both spermatogonia and oögonia. The nucleus is filled by a mass of dense, granular karyoplasm, which stains readily with plasma dyes, and through it runs a delicate achromatic reticulum, radiating from the nucleolus (Fig. 3) and bearing at its nodes a large number of small thickenings or karyosomes. The nuclear membrane is thick, stains darkly, and is apparently of granular nature. When treated with the Auerbach mixture of acid fuchsin and basic methyl green, the cytoplasm, nuclear membrane, karyoplasm, reticulum, and karyosomes select the red, acid dye; the outer shell of the nucleolus alone selecting the basic dye, while its central plasmosome usually appears of a dull brownish tint. The nucleolar shell thus presents the acid reaction typical of chromatin in mitosis, while all other portions of the cell are now basic in this respect.

The first change in the prophase is a thickening of the threads of the reticulum and an increase in the size and staining capacity of the karyosomes (Fig. 4). The karyoplasm now loses its granular appearance and

becomes less and less dense, until it finally disappears altogether, leaving the nucleus wholly translucent and apparently empty, except for the reticulum and nucleolus, although we must assume that it is still filled with some fluid substance. We cannot reach any very definite conclusions as to the actual cause of the disappearance of the karyoplasm, yet, from the radical nature of the alteration, we must assume that it is the visible expression of profound metabolic changes in the general constitution of the nucleus. Furthermore, it is accompanied by one very significant event: the karyosomes, previously basic, now reverse their reaction and attain the acid character typical of chromatin in mitosis, as is shown by their selecting the basic dye in the various acid-basic mixtures.

I may anticipate the following account by stating here that the same two changes are likewise precisely contemporaneous in every other mitosis which I have studied in Gonionemus. This is a very striking and suggestive phenomenon; but I am not prepared to conclude from it that the reversal of the staining reaction of the karyosomes is a result of the dissolution of the karyoplasm. It is perhaps more probable that both are manifestations of the same underlying modifications of the nuclear protoplasm.

The reticulum now lies chiefly in a peripheral position, in contact with the nuclear membrane, though many of its strands originate from the substance of the nucleolus, which is still nearly central (Fig. 4). In the latter structure, which has hitherto preserved the condition already described for the "resting nucleus," marked changes now take place. In the stage shown in Figure 5 it has lost its sharp contour, and bears at its margin a series of irregular masses, which, to judge from their staining properties, are of chromatic nature, — one at the origin of each of the linin strands. There is no doubt, I think, that these structures have been derived from a partial disintegration of the deeply-staining chromatic shell of the nucleolus, and that the central mass (Fig. 5), now less deeply stained, is to be regarded as equivalent to a plasmosome. Later stages in the disintegration of the nucleolus are most easily traced on crushed cells, where the parts of the nucleus are separated. Such a specimen, in which, however, only a few of the karyosomes are shown, is represented in Figure 7. The nucleolus now consists of a central vacuolated plasmosome, with six or seven separate chromatin masses attached to its margin at the points of origin of as many linin strands. At a slightly more advanced stage (Fig. 6) these masses become wholly detached from the plasmosome, which then disappears. The clearness

with which these successive stages can be followed, together with the evidence afforded by the staining reactions of the nucleus, seem to me to show beyond reasonable doubt that the nucleolar shell does actually contribute to the formation of the chromatin reticulum, and thus, indirectly, to the chromosomes. The evidence indicates that the chromatin substance which forms the peripheral portion of the nucleolus does not consist of discrete structures, but is rather to be regarded as a granular mass of viscid consistency, which breaks up into irregular portions in the prophase.

As the chromatin shell of the nucleolus disintegrates, the karyosomes become correspondingly larger and at the same time denser, and more sharply outlined (compare Figures 4 and 7); instead of being situated only at the nodes of the reticulum, they now lie largely along the course of its threads. This is of course further evidence for the view that, with the breaking down of the nucleolar shell, separate masses of chromatin become disengaged and then migrate outward along the course of the linin strands. I believe that the karyosomes at this stage (Fig. 6) are no more to be regarded as discrete and individual chromatin bodies than are the chromatic masses derived from the nucleolar shell, but that they are merely aggregations of chromatic microsomes which have gathered at different spots on the achromatic strands. This assumption is supported by the fact that in different nuclei they vary greatly in size, outline, and composition (being either dense or granular), as well as in number.

The karyosomes now arrange themselves more and more evenly along the courses of the strands, while the net as a whole becomes looser, apparently through the breaking down of some of its meshes (Fig. 6). This process of condensation of the chromatin masses finally results in the formation of definite segments, which, from their composite origin, resemble roughly a string of beads. These segments are entirely independent of one another so far as their chromatin is concerned, but they are connected by the persistent linin strands, which form their basis.

This seems to be the nearest morphologic approach to a spireme that is found in the somatic cells of Gonionemus, for I have not been able to find any evidence that the net ever becomes metamorphosed into a single continuous thread, such as Downing (:05) observed in the interstitial cells and male germ-cells of Hydra fusca. I have never seen a continuous spireme in any prophase in Gonionemus, although I have searched for it diligently, and although my material for the study of the spermatogonial and spermatocyte divisions is very abundant.

The number of karyosomes going to make up each segment is not constant, but varies from two to four or five (Fig. 6). This is another reason for believing that the karyosomes have no individual significance. It has proved to be impossible to count the chromatin segments with absolute accuracy; yet, from such counts as I have been able to make, there is little doubt but that the number is twenty-four. This (Fig. 6) is the latest stage in which the plasmatic portion of the nucleolus can be demonstrated. Although there is little question of the plasmatic nature of this body, it shows great individual variation in its reactions to iron haematoxylin, sometimes staining intensely, at other times, even with apparently the same treatment, hardly at all.

It is difficult to trace the stages in the formation of the chromosomes, because after the breaking down of the nuclear membrane the entire reticulum becomes very much contracted. Certain phases of the process are, however, clearly indicated. The membrane, distinct and staining deeply, persists until shortly after the formation of the chromatic segments, and then disintegrates, the nucleolus also disappearing at the same time. Cytoplasm now takes the place of the transparent fluid formerly filling the nucleus, and in it the reticulum lies in a contracted condition (Fig. 8). This sudden change from the apparently inert nuclear sap to the much denser cytoplasm is undoubtedly of importance in the later history of the nuclear elements. The segments now appear denser and more deeply stained (Fig. 8); it is probable that this condensation leads to the formation of the chromosomes, without any further process of segmentation, so that the chromatin-segment stage may thus be regarded as ontogenetically equivalent to the segmented spireme of the higher Metazoa (see Fig. 8, which shows in a single nucleus several stages in the process of condensation). Each chromosome when fully formed exhibits a double or dumb-bell form, a condition found also in the spermatogonia. Here however, each chromosome is formed by the union of two chromatin masses, the chromomeres of Downing (:05). In the entoderm cells I have found no evidence that such a union takes place, the method by which this peculiar form arises being here less apparent. The chromosomes still retain their connection with one another through the linin strands. They now commence to take up their positions in the equator of the cell, and this is the most favorable opportunity for counting them. Even now, however, they are usually much crowded, so that an error of one or two is always possible; yet from an examination of several favorable cases I believe that the number is in all probability twenty-four. Two such

cells are represented in Figures 9 and 10, both of which afforded a comparatively accurate count; they also show the dumb-bell form of many of the chromosomes, which is usually if not always lost in the later metaphase. In the equatorial plate the chromosomes lie with their long axes parallel to the future plane of cell division, and therefore when seen endwise present the aspect of minute circular bodies (Fig. 11). Longitudinal splitting now takes place, and the daughter chromosomes begin individually their migration toward the poles of the spindle; frequently they retain their position transverse to the axis of the spindle (Fig. 11); but usually they revolve, so as to lie parallel to the axis of the spindle. As I have already stated, in the description of the resting stage, no archoplasmic structures or centrosomes are then to be seen, nor do any such appear until the formation of the equatorial plate. The spindle figure can then be traced, and at each of its poles is visible a minute granule, the centrosome, without any trace of a surrounding sphere. As to the origin of this body I am wholly in the dark, nor can I offer any evidence as to its fate, except that in the early anaphase it can no longer be demonstrated. The entire achromatic figure is extremely simple, consisting in the metaphase merely of the centrosomes and spindle fibres, without any trace whatever of astral radiations. The spindle fibres are very delicate, even in vom Rath preparations, and do not appear to be of granular nature; but with the separation of the chromosomes there are formed interzonal filaments, which are very much thicker than the spindle fibres and are clearly formed of rows of distinct granules (Plate **2**, Fig. 12). During the migration of the daughter chromosomes, which usually takes place in an irregular manner, one or two outstripping the others, the polar portions of the spindle fibres grow less and less apparent, while the interzonal filaments become even thicker, presenting, particularly in cells fixed in the vom Rath fluid, the appearance of very definite rods, often bent outward somewhat at the centre, as is shown in Figure 12. Finally, as the chromosomes closely approach the poles (the centrosomes are now no longer to be seen), the polar parts of the fibres disappear altogether, leaving in their place merely a small homogeneous mass of archoplasmic substance (Fig. 12); the interzonal filaments, on the contrary, persist until after the constriction of the cell is completed (Fig. 13). Certainly, then, these cells afford no evidence whatever in support of the well-known theory of cell division through fibrillar contraction; and we must look to some agency other than that of a contraction of the spindle fibres to effect the migration of the chromosomes. Furthermore, there can, I think, be no doubt that spindle fibres and interzonal filaments are in this case structures of very diverse nature.

As the chromosomes approach the poles they become very closely crowded together (Plate **2**, Fig. 12), but at a slightly later stage they separate once more and are now seen to be connected, probably at their previous points of contact, so that they form an irregular network (Fig. 13). At the same time the new nuclear membrane is formed in each daughter cell, and constriction of the cell body takes place. Up to this time the daughter nuclei remain connected with each other by the bridge of interzonal filaments (Fig. 13), but these now break down and are absorbed by the cytoplasm. With the further regressive metamorphosis of the nucleus the individual chromosomes disintegrate, so that they are no longer distinguishable from one another, and the chromatic net becomes denser and more homogeneous. The latter then breaks down into its minute karyosomes, which are connected by a delicate linin reticulum and finally become dispersed throughout the nucleus (Fig. 14). This stage is especially interesting from the light which it throws on the question of the origin of the nucleolus. The two cells represented in Figure 14 show the earliest stage at which this structure is distinguishable from the karyosomes. It is now very small, hardly larger than the latter, dense, and homogeneous, and in its staining properties, indistinguishable from chromatin. From its structure at this stage it seems to me probable that it originates as the result of the coalescence of two or more karyosomes, just as it certainly does in the case of the primary spermatocytes (page 311). During its growth it retains its homogeneous character, and it is not until it reaches its mature size (Plate **1**, Fig. 3) that it shows any differentiation of its substance. There can be little doubt, then, that the nucleolus of the somatic cell in Gonionemus shows a very close genetic relationship to the chromatin net; or that its plasmatic central portion arises as a differentiation or growth-product of the primitive chromatic mass.

### B. Spermatogenesis.

The gonads of Gonionemus consist of a complex series of folds of the oral surfaces of the radial canals, occupying the distal two-thirds of their length. A cross section of the male gonad (Plate **1**, Fig. 2) shows a mass of sex cells which, as might be expected, are developed in the ectoderm, and represent the several generations commonly described in the spermatogenesis of other animals. Of these the spermatogonia lie basally or next the mesogloea, while the more advanced generations occupy a more superficial position. The arrangement, however, is rather irregular.

### 1. *The Spermatogonia.*

The spermatogonia, which, in young gonads, form a layer next the mesogloea four or five cells deep, are at first hardly distinguishable from ordinary ectodern cells; but after a period of multiplication, resulting in an undetermined number of cell generations, they enter upon a period without multiplication, from which they emerge much increased in bulk. The ordinary ectoderm cells measure about 5.5 $\mu$ in diameter and their nuclei 3 $\mu$, while the largest spermatogonia and their nuclei are respectively 10.5 $\mu$ and 8.5 $\mu$ in diameter. The amount of growth is, however, very variable (Plate **2**, Figs. 15 and 18), depending, it seems, chiefly on the position of the cells in the gonad and the readiness of their access to a supply of food. That the different sized cells are existing under different metabolic conditions is shown by the condition of the cytoplasm, which contains in the larger cells many amorphous and non-vital structures, which are seldom or never found in the smaller. The larger cells in general appearance recall, in a striking way, the oögonia and early oöcytes. When closely packed together their outlines are roughly polygonal, but when lying in more isolated positions, they readily assume a more oval outline, showing that variability of form which is so characteristic of coelenterate cells. The nucleus (Fig. 15), which has more than half the volume of the cell, is spheroidal, bounded by a well-defined membrane, and encloses one, or sometimes two, large nucleoli. The remainder of its area is filled with a rather dense and distinctly granular karyoplasm, through which is to be traced an irregular and exceedingly delicate linin reticulum, bearing at its nodes distinct karyosomes. Many of the strands of the net extend outward from the substance of the nucleolus (Figs. 15, 17), which is very large. After treatment with iron haematoxylin it sometimes appears, as in Figure 16, densely stained and homogeneous, but in the majority of cases (Figs. 15, 17, 18) it shows a pale, even transparent, central area surrounded by a deeply stained "shell." Occasionally, perhaps as the result of faulty manipulation, this central region becomes highly refractive and sharply outlined, but I am convinced that such appearances are artificial. It is, of course, evident that we are dealing with a structure of the same nature as that already discussed in the case of the endoderm cells; that is, here also the nucleolus is of compound nature, consisting of a central sphere or plasmosome, surrounded by a shell, which is shown by later changes to be of chromatic nature. This condition of the nucleolus, while realized in somatic cells and oögonia as well as in spermatogonia,

differs sharply from that seen in the spermatocytes, and forms, therefore, an excellent criterion for determining whether any particular cell in the resting stage is a spermatogonium or a spermatocyte. Size, although a valuable index, is in this question much less reliable; and position, whether close to the mesogloea or more peripheral, is not a safe indication, since groups of cells in different parts of the gonad develop at different rates.

The cytoplasm of the spermatogonia stains strongly with plasma dyes, is densely and evenly reticulate, contains no vacuoles, and shows no archoplasmic structures that are clearly demonstrable. In fact, in no medusan cell in the resting stage (except, of course, the spermatid) have I been able to distinguish idiozome, Nebenkern, or other permanent cell organ of similar nature. The question of the presence or absence of the centrosome is, however, less easily answered. In a considerable number of cells (Fig. 19) I have been able to distinguish near the nucleus a minute black spherule, frequently surrounded by a clear area, in which the reticulate appearance of the cytoplasm is not visible. At first sight this body might well seem to be the centrosome, but it is by no means certain that such is the case. In many cells (Fig. 16) there is visible a black granule which, however is surrounded by undifferentiated cytoplasm. In the great majority of cells no such structure is to be seen, while, on the other hand, the cytoplasm sometimes contains several dark granules of different sizes (Fig. 16). Furthermore, the presence or absence of the homogeneous area above mentioned seems to be of little significance, for I have often observed similar appearances at the periphery of the large metaplasmic masses to be described presently. Finally, I have not been able to trace with certainty a genetic connection between any of these structures and the centrosomes first demonstrable beyond doubt in the early metaphase of division. It might be reasonably supposed that the sure detection among the various cytoplasmic granules of so minute a body as the centrosome would be very difficult. It should, however, be borne in mind that in the spermatid, as later described, there is no stage when the centrosome is not clearly visible, even though it is very small and imbedded in cytoplasm of the same granular appearance as that of the spermatogonia. We must conclude, then, that the presence of a centrosome in the resting stages of the spermatogonia is at best doubtful, and if we assume its existence it must be on *à priori* grounds rather than from any actual internal evidence afforded by the cells. I lay stress upon this for the sake of emphasizing the difference exhibited by somatic cells, spermatogonia, and spermatocytes on the one hand, and spermatids and spermatozoa on the other.

Although the cytoplasm does not exhibit any archoplasmic structures, it does, in the case at least of the larger cells, contain a greater or less number of deeply staining masses which are of various sizes, homogeneous structure, indefinite outline, and lie as a rule close to the nuclear membrane (Plate **2**, Figs. 15, 17, 24, 27). These bodies blacken with osmic acid, and after being bleached with chlorine combine strongly with acid dyes. In appearance and general structure they are evidently closely allied to certain structures found in the younger oöcytes of the same species (see page 341 and Plate **5**, Figs. 109, 115; Plate **6**, Fig. 125), and to some of the various metaplasmic objects in other eggs which, in spite of their diverse natures, have been grouped together under the name of yolk nuclei. Although these structures first appear in contact with the nuclear membrane (Fig. 1), there is no evidence that they are actually nuclear products. But we must, I think, conclude with Crampton (**'99**), and E. B. Wilson (: **00**) that they are of cytoplasmic origin, formed, however, under the influence of the metabolic activity of the nucleus. Eventually, during the prophase of the last spermatogonial mitosis, they gradually dwindle and disappear.

The staining reactions, which afford the best available index to the chemical conditions of the different cell structures, are of interest. In the resting spermatogonia, after treatment with the Auerbach mixture, cytoplasm, metaplasm, nuclear membrane, karyoplasm, karyosomes, linin reticulum, and sometimes the central portion of the nucleolus, select the acid (red) dye, the only nuclear structure selecting the basic dye being the nucleolar "shell." I do not, however, believe that this is conclusive evidence that this shell now contains all the chromatin which is later visible, since the later stages show beyond doubt that the karyosomes contribute to it.

*Prophase.* — At the close of the growth period the cells enter upon the prophase of the last spermatogonial mitosis. In general this resembles the somatic mitosis already described; yet differs in certain stages of the prophase, especially in the mode of formation of the definitive chromosomes. The first apparent change affects the achromatic network, which becomes stouter, while the karyosomes lying at its nodes increase in size and staining capacity without, however, as yet changing their chemical reaction (Plate **2**, Figs. 19, 20). It is now more evident than in the resting stage that many of the achromatic strands radiate outward from the nucleolus. The chromatic shell of the latter loses its continuity and presents the appearance of numerous distinct masses, a change which is contemporaneous with a marked increase in the stout-

ness of the reticulum and the size of its karyosomes (Fig. 20). The chromatin masses surrounding the nucleolus decrease in number until it is clearly visible that they are attached to the periphery of a distinct plasmosome (Fig. 20), and that each one lies at the point of origin of one of the linin strands. This change is accompanied by a progressive increase in the thickness and staining capacity of the strands; and the evidence seems to show that the chromatic granules of the nucleolar shell migrate outward along the course of the achromatic fibres. At about this time the karyoplasm disappears, leaving the nucleus filled merely with transparent fluid, and simultaneously it is seen, on Auerbach preparations, that the karyosomes have changed their chemical reaction, being now no longer basic but acid. This change is exactly the same that occurs in somatic cells at a similar stage, and has already been discussed (see page 32). The spermatogonium now presents the appearance represented in Figure 21. The network is stout, while the number of meshes appears to have decreased somewhat; a varying number of chromatin masses are still attached to the periphery of the nucleolus, while the karyosomes lying at the nodes stain strongly with iron haematoxylin, and are more prominent and sharply outlined. Their number is very considerable, but seems to vary somewhat in different cells; and since they vary also greatly in size, outline, and composition, being either dense or granular, I believe that they do not represent discrete chromatin structures, but merely aggregations of chromatic microsomes collected along the course of the achromatic network. In many ways they recall the appearance and behavior of the very much more numerous karyosomes seen in the nuclei of oöcytes in the latter part of the growth period, which afterward fuse to form the chromatic segment (see page 346–347).

The karyosomes now become massed along the net strands, thus forming definite chromatic segments, which are separate from one another, yet stand in connection through the persistent linin strands (Figs. 20, 22). At first, as in the cell shown in Figure 20, there may be only one or two such segments; but they increase in number until eventually the entire net consists of such beaded segments. I have been unable to determine that there is an invariable number of segments, while the number of karyosomes taking part in the formation of each segment appears to vary from two to four or five. The fact that these changes are not precisely synchronous in all parts of the same nucleus is a great help to their true interpretation, for, as is the case in the cell represented in Figure 20, several successive stages — in this case, both sepa-

rated karyosomes and beaded segments — are often represented in its different regions.

By the time the chromatin is massed in the form of "segments," most of the net strands have lost their connection with the nucleolus (Fig. 22), and the segments have taken a peripheral position close against the membrane, leaving the central area of the nucleus vacant. This condition of the nucleus is seen particularly well on isolation preparations, when, by focussing at the equatorial level, it appears as a clear circular area, surrounded by a ring of black dots, the segments in optical section. It will be recalled that in the entoderm cells these become condensed to form the chromosomes directly, and at first I believed the same to be true of the spermatogonia as well. More attentive study has, however, convinced me that this is not the case, but that here they either segment or condense into numerous chromatin bodies; the result being such a stage as is represented in Figures 23 and 24. These bodies are larger than the original karyosomes, stain deeper, are nearly spherical, sharply outlined, and lie, like the parent segment, close against the nuclear membrane. From their clear-cut appearance it is easy to make an approximate count of them, and in all favorable cases I have found from 48 to 51 or 53. In all probability their true number is exactly twice that of the somatic chromosomes, that is, forty-eight. These chromomeres — as we may call them, following Downing (: **05**), who finds in Hydra a condition essentially similar — still remain connected together by delicate linin strands, which never form a single continuous thread, but retain the "net" structure of earlier stages. This process of concentration, like the formation of the beaded segments, does not always occur synchronously throughout the entire nucleus, but often, as in the cell represented in Figure 23, some of the segments may persist; in this case one such, *x*, is to be seen. The nucleolus, now of course a purely plasmatic structure, and still connected with some of the achromatic threads, has lost most of its stainability, and after this stage either fragments or is absorbed. At any rate it can be traced no further.

In the study of this stage, cells so crushed that the membrane is partly destroyed and the nuclear contents more or less isolated are of great assistance in indicating the degree of independence of the different structures. Such a cell, crushed by the cover glass, is represented in Figure 25, and shows several distinct stages. The nucleolus still persists; in the upper portion of the figure within the nucleus, where the nuclear membrane still persists, are to be seen chromatic segments; at the lower side are three or four more, and a considerable number of chromomeres

in essentially the condition shown in Figure 23. Furthermore, there is, at *x*, a single dumb-bell-shaped chromosome fully formed.

Changes now occur resulting in the destruction of the nuclear membrane, the formation of the chromosomes, and of the achromatic spindle figure. The actual steps in the formation of the chromosomes are traced only with great difficulty, owing to the fact that with the disappearance of the membrane, the chromatin structures become as a rule so densely crowded together that, although stages in the process are fairly common, it is difficult to correlate them. Fortunately, however, in a few cases I have been able to find cells at this stage whose nuclear contents were more loosely arranged, and one such is represented in Figure 26. The nuclear membrane has wholly broken down, and the former nuclear area is occupied by a substance which by its homogeneous or slightly granular nature is readily distinguished from the surrounding cytoplasm. The chromatic structures present a condition not essentially different from that seen (Fig. 23) just prior to the disappearance of the membrane, and consist of a considerable number, probably forty-eight, spherical, sharply outlined and deeply stained chromomeres connected with one another by a loose and irregular network of linin strands. The occurrence of cells exhibiting this condition is very strong evidence for the view that the condensation of the chromatic segments into chromomeres twice as numerous as the chromosomes, and not directly into the chromosomes themselves, is a normal and typical step in the mitotic process of the spermatogonia. The formation of the definitive chromosomes, which, in the early metaphase, are dumb-bell-shaped structures, probably takes place by the conjugation of adjacent pairs of chromomeres. Figure 27 represents a stage in this process, several of the chromosomes showing the mature condition, while many of the chromomeres are still independent.

This type of chromosome formation, which differs very markedly from the process occurring in somatic cells and in oögonia, agrees in general, as we shall see later, with the course of events in the first generation of spermatocytes, except for the number of the bodies involved. Yet the ensuing mitosis results, as it does in oögonia and somatic cells, in an equatorial division of chromosomes in the somatic number.

The succession of stages leading from the resting nucleus to the chromatin-segment (modified spireme) stage, is so close and the conditions are so clear as to allow of but one interpretation. Unfortunately this is not the case with the processes involved in the formation of the chromomeres from these segments. Indeed, were it not for the later stages, it

might be difficult to say which of these conditions preceded the other, since there is in the cell itself no definite landmark, the disappearance of the nucleolus occurring at different periods in different cells. When, however, we find that after the disappearance of the nuclear membrane the chromatin is still present in the form of chromomeres, and that these later fuse to form half as many chromosomes, only one interpretation of the method of chromosome formation is open to us. It is, I think, clear that the "segment" stage of the prophase of the spermatogonia of Gonionemus is not homologous with the segmented spireme of higher Metazoa, although in general aspect it may resemble it, but rather with the spireme itself, which here fails to form a strand of continuous thickness throughout.

The protoplasmic mass which occupies the position of the nucleus after the disintegration of the latter is, as already noted (Fig. 26), distinctly granular and clearly distinguishable from the surrounding cytoplasm, and since it is in this mass that the spindle rays later develop, we may fairly assume that it is of archoplasmic nature. But whether it consists of persistent archoplasmic granules previously masked by the cytoplasm, and now assembled preparatory to the rôle they are to play in the ensuing mitosis, or whether it merely represents ordinary cytoplasm altered chemically and morphologically through its contact with the nuclear structures, can not be certainly answered. No doubt very considerable changes in the surrounding cytoplasm must occur at the time of the disappearance of the membrane, changes affecting both its composition and the degree of local concentration of the colloid substances of which the cell is composed. Although I do not wish to enter here upon any discussion of the much mooted question of the mechanics of cell division, yet such alterations cannot fail to be suggestive, especially in view of the more recent theories of the influence of electrodynamic forces in determining the arrangement of the chromosomes in the equatorial region of the cell.

*Metaphase.* — After the formation of the dumb-bell-shaped chromosomes, these rapidly take up a position in the equatorial region of the cell, lying with their long axes parallel to the future plane of division, so that, when the cell is viewed from the side they appear as small intensely black dots (Fig. 31). The chromomeres have now become so closely fused that the double outline is usually lost; and they are rod-like (see Figs. 28, 29 and 30). As soon as they reach the equatorial-plate stage, longitudinal splitting commences, which makes it difficult or impossible to arrive at an accurate count of chromosomes in the early

metaphase. Since, however, in all the earliest cases studied, I have found from 25 to 28 or 29, and a few of these invariably lie at a different level from the others, I believe there is no reasonable doubt that the number is actually the same as in somatic cells, in all probability twenty-four (see page 299, and Figs. 9, 10). As the splitting progresses (Figs. 11–13), all the daughter chromosomes are usually visible in pole views, so that with the advance of the process the apparent number constantly increases, a condition which at first, when I endeavored to determine the relation of the spermatogonial to the somatic mitoses, proved very embarrassing. The process of splitting is parallel to the future plane of cell division and thus longitudinal; it commences first at one end of each rod-like chromosome, so that there constantly occur V-shaped figures, many of which are seen in the cells represented in Figures 29 and 30. From what we have learned of the structure of the chromosomes of this animal, it is evident that the split must divide each chromomere into two, and that it is therefore truly equational, not only for the chromosome but for its component parts as well.

It is in the early metaphase that the achromatic figure can first be clearly distinguished (Fig. 31). This, as described by both Aders (:**03**) and Downing (:**05**) for Hydra, is exceedingly simple, its most important feature being, as in the case of the somatic cells, the entire absence of any trace of astral radiations.

The centrosomes, which, as already noted, cannot be demonstrated previous to the metaphase, are exceedingly minute granules (Fig. 31), lying directly at the focus of the spindle rays, without any trace of centrosphere, and situated either at the margin of the cell (Fig. 31) or some distance within its substance (Plate **3**, Fig. 32). Preparations fixed with Flemming's mixture show characteristic differences in the appearance of the spindle from those fixed in vom Rath's fluid. In the former case its ground substance appears homogeneous, staining more darkly than ordinary cytoplasm; the fibres are exceedingly delicate and can be traced only with difficulty. In the latter it presents no difference from cytoplasm; the fibres, however, are much thicker, stain darkly, and are plainly composed of series of apposed granules; the centrosomes are larger and more prominent. These differences, moreover, are characteristic of the achromatic figures of all the mitoses studied.

*Anaphase.* — The daughter chromosomes now commence their migration toward the poles; this, however, is not, as a rule accomplished by all the chromosomes simultaneously, for one or two usually outstrip the others (Plate **2**, Fig. 31). They may still retain a position parallel to

the plane of division, but more frequently they revolve so as to lie parallel to the spindle rays, and it is now seen that they once more exhibit the dumb-bell form temporarily lost during the metaphase. In cells partly crushed the condition of the chromosomes appears most distinctly (Fig. 32). Occasionally, when the migration is more nearly simultaneous, typical daughter plates are formed, and in polar views of several such I have been able to count the chromosomes with comparative accuracy. Thus, in the cell shown in Figure 34 *a* and 34 *b* (Plate 3), viewed somewhat obliquely, I was able to count in one daughter plate 24 chromosomes, and in the other apparently 25. This, of course, is additional evidence that in the spermatogonia the chromosomes are present in exactly the same number as in the somatic cells. As they continue to approach the poles, interzonal filaments are formed which appear, especially in vom Rath preparations, much stouter than the spindle fibres, a condition characteristic of all mitoses in adult tissues of Gonionemus (Fig. 35). With the further migration of the chromosomes, the polar portions of the spindle fibres gradually dwindle, and finally seem to disintegrate (Figs. 35, 36), though leaving the conical mass of archoplasmic substance focusing at the centrosome still sharply marked off from the surrounding cytoplasm. The interzonal filaments, on the other hand, increase in stoutness with the progress of cell division. This is, of course, exactly the opposite of what we should expect if we were to explain the process of mitosis on the theory of fibrillar contraction.

Constriction of the cell body now takes place (Figs. 35–38), but the two daughter cells long remain connected by the bridge of interzonal filaments, which bear at their central point a series of distinct Zwischenkörper. Eventually, however, this bridge dwindles and breaks down (Figs. 39, 40), and the remnants of the fibres disappear. The centrosome cannot be traced after the late anaphase; and its fate is doubtful, for with the disappearance of the spindle remnants there is no longer any index to its position, and it easily becomes indistinguishable among the cytoplasmic microsomes.

During the anaphase the chromosomes often become very closely crowded (Fig. 36), even appearing to fuse, an event described by Downing (:05) as occurring in Hydra. In Gonionemus, however, this is not the case, for by careful focusing it is always possible to resolve the mass into separate chromosomes, which now, however, have lost their sharp outlines and are of a more or less granular consistency (Fig. 38).

The re-formation of the nucleas appears to be essentially like the process described by Lerat (:05) in the case of Cyclops. The chromo-

somes move somewhat apart once more, and it is seen that they have become connected to form an irregular network (Fig. 38). There is in Gonionemus no evidence that the achromatic strands persist throughout mitosis, as described by many investigators. I believe the true explanation is that the chromosomes, while closely in contact, become more or less confluent. It must be borne in mind, however, that every chromosome is, no doubt, of mixed origin, chromatic and achromatic, and that the connecting links of the reticulum may thus be formed chiefly, if not entirely, from the achromatic portion of the chromosomes. The net thus formed encloses between its meshes a large amount of cytoplasm. The nuclear membrane now reforms, and the enclosed cytoplasm probably becomes the karyoplasm of the spermatocyte nucleus.

The threads of the reticulum become more and more homogeneous, until finally the individual chromosomes are no longer distinguishable (Fig. 37). The nucleus, till now much flattened and lying near the periphery of the cell, becomes oval in outline and takes up a more nearly central position. It increases in size, and at the same time the network becomes looser, and finally disintegrates, its place being taken by delicate linin strands, while the chromatin breaks down into numerous microsomes. The reformation of the nucleolus takes place through the aggregation of certain of the chromatic microsomes (Fig. 39), just as has already been described in the case of the mitosis of the entoderm cells (page 301). But in the present case stages in the process are so much more numerous that the actual course of events can be established more certainly. The cells now enter upon a period of apparent quiescence, preparatory to the prophases of the first maturation division. Cells of this stage are usually designated according to the terminology introduced by Boveri in 1891, as the last generation of spermatogonia. I prefer, however, to term them already the primary spermatocytes, for it seems to me more logical to adopt names based on the cell generations rather than on conditions of growth.

### 2. *The Primary Spermatocytes.*

The spermatocytes of the first generation are, during the resting stage, much smaller than their parent spermatogonia, the diameter of the entire cell measuring only about 8.5 $\mu$ and that of the nucleus 6.5 $\mu$. Spacial relations within the gonad are of some assistance in determining the cell generations, since the spermatogonia, except in a few isolated cases, lie basally, in contact with the mesogloea, whereas the primary spermatocytes in various stages form a broad zone just peripheral to them, and

the secondary spermatocytes, spermatids, and spermatozoa constitute the outermost layer (Fig. 2, which shows a cross section of a gonad).

The so-called resting stage of the cells under consideration appears to persist for a considerable period, since cells in this condition are very abundant; but there is little or no growth connected with it; in this it differs markedly from the corresponding phases of both the spermatogonia and the female germ cells of Gonionemus, as well as from the conditions of the cell generations so commonly described by other authors among higher Metazoa, particularly arthropods (cf. especially Blackman, **:05**).

The nucleus (Plate **3**, Fig. 40) is oval; its membrane, which is distinct and granular, stains strongly with acid dyes; the karyoplasm is dense; the achromatic reticulum, many of whose strands take their origin in the substance of the nucleolus, is very delicate and bears at its nodes small thickenings, the karyosomes. I have been able to make a more thorough study of the karyoplasm in this than in the other cell generations, particularly in crushed preparations, and with the following results. As far as its chemical reactions are concerned, it stains but slightly, even after prolonged chlorine bleaching, and then only with acid dyes, not at all with basic ones, presenting in this a sharp contrast to the corresponding substance in the oöcytes, which with the same treatment show a very strong affinity for plasma stains. After treatment with osmic acid it forms a solid and very brittle mass, which, when forcibly extruded from the nucleus, breaks into angular and irregular fragments, presenting a characteristic granular appearance very different from the reticulate cytoplasm surrounding it. Finally, after treatment with Flemming's fluid, it is, at this stage, of a peculiar yellowish color seen in no other part of the cell. Since there is little doubt, from its previous history, that it is derived from ordinary cytoplasm, we must suppose that it has undergone these modifications under the influence of the metabolic processes of the nuclear structures.

The nucleolus is much smaller, both actually and in proportion to the size of the cell, than in resting spermatogonia (cf. Figs. 40 and 41 with Figs. 15 and 16), and, as shown by its origin and fate, is a purely chromatin structure, a feature previously noted by Guenther (**:04**) in spermatocytes of Hydra viridis. It is, as previously noted, a precise criterion for distinguishing cells of these two generations. Usually it is single; occasionally, however (Fig. 41), there are two, both of normal size, in which case each acts in the prophase independently of the other. The cytoplasm when compared with that of spermatogonia is rather scanty, and presents

the same finely reticulate appearance as in the earlier generation. I have been unable to detect any archoplasmic structures whatever, nor is the presence of a centrosome to be demonstrated with certainty, although minute granules are often found which might be supposed to represent that structure. The cytoplasm does occasionally, however, contain small metaplasmic masses similar to those described in the last cell generation (see page 304, and compare with Figs. 15 and 17), though they are here much smaller (Fig. 40), and often not to be found at all. When present, they are closely apposed to the nuclear membrane, and disappear during the prophase. The staining reactions of the cell closely parallel those of the spermatogonia. With the Auerbach mixture, the best microchemical test easily available for fixed material, the nuclear membrane, reticulum, and karyosomes select the red (acid) dye, while the nucleolus stains a very pure green, which of course indicates that it is very rich in nucleinic acid.

### 3. *The First Maturation Division.*

*Prophase.* — The early prophase of the first maturation division closely resembles that of the somatic and spermatogonial mitoses. The karyosomes increase in size, and become denser and more sharply outlined, and it is evident that hand in hand with their increase in bulk the nucleolus suffers a corresponding decrease in volume until it finally disappears entirely (Figs. 42 and 43, which represent two successive stages in this process). The prophase, then, is in so far a reversal of the telophase of the last spermatogonial division, as the nucleolus, then formed through the coalescence of numerous chromatin masses, now disintegrates through their dispersal. Guenther (:04) was able to find in the spermatocytes of Hydra all stages from such a nucleolus to the definitive chromosomes; but he gives no figures of the process, nor does he describe it in detail. Downing (:05), on the other hand, makes no mention of any nucleolus in primary spermatocytes of Hydra.

Shortly before the disappearance of the nucleolus, the karyosomes reverse their staining reaction, now exhibiting the characteristic affinity of chromatin for basic dyes — a change which is nearly simultaneous with the disappearance of the karyoplasm. The nucleus is now (Fig. 44) filled with a complicated reticulum, which bears at its nodes and along the course of its meshes a great number of small chromatin masses, the karyosomes. I have not been able to count these accurately, nor do I believe that their number is important. On the contrary, I regard it as probable, from their variations in size, that they also vary in

number. As they now increase still more in bulk, and at the same time diminish in number, it is probable that adjacent karyosomes coalesce (Figs. 45, 46), while the strands of the network show similar changes, becoming thicker and less numerous and staining more deeply. This process of coalescence on the part of the karyosomes, and thickening on the part of the strands, continues until finally there results, after the several successive stages shown in Figures 44–46, a continuous and homogeneous net composed of a comparatively small number of thick, smooth, and very strongly staining strands, often with thickenings at the nodes, as is shown in Figure 47. Fortunately, these changes do not always take place at precisely the same time, so that several stages are often to be seen in different parts of a single nucleus (Fig. 46). As the cells are exceedingly abundant and, from the clearness of their nuclei, easily studied on isolation preparations, it has been possible to follow the process in detail. This stage, as shown by its earlier history and fate, corresponds exactly to the "chromatin-segment" stage seen in the spermatogonia, and hence both are to be regarded as the homologs of the "spireme" of other forms. Cells of this stage show great variation in the number and arrangement of their meshes; in some cases, as in Figure 47, these are comparatively simple, while in others, as in Figure 49, they are so complicated and interlocked that it is impossible to resolve them. But they all agree in showing no trace of a "beaded" appearance, and we may therefore assume the union of the chromatin granules to be extremely intimate. The time necessary for these changes must be considerable, since a great majority of the cells in all the preparations examined exhibit some of the above described stages, and since these changes are accompanied by a decided diminution in the bulk of the cytoplasm (compare Figs. 40 and 47).

We now come to a consideration of one of the most difficult, and at the same time one of the most important, stages in the whole spermatogenesis of Gonionemus, that of the "pseudosynapsis." The unravelling of the processes leading from the spireme to the formation of the chromosomes in the metaphase of the first maturation division has proved exceedingly difficult, and even now, after careful study, I am only too well aware that my interpretation may fairly be questioned. As is well known, the majority of students of spermatogenesis have described a stage occurring at some period between the last spermatogonial and the first maturation divisions, in which the chromatin strands become more or less densely massed at one side of the nucleus. This was first observed by Moore ('95), and, following him has usually been termed the stage

of synapsis; moreover it has been maintained by most authors that it is during this stage that the numerical reduction, or pairing, of the chromosomes takes place.

Such appearances occur in Gonionemus in great numbers among the primary spermatocytes (Figs. 50–54), and at first I had little question that I was dealing with a true "synapsis" zone. Further study, however, threw doubt on this conclusion, and I may at once state my conviction that, in the present species at any rate, they are purely accidental, and in no way connected with the essential features of synapsis. This may seem rather a sweeping statement, but it is, I think, supported by all the facts of the case. The starting point in the formation of such artifacts is represented in Figure 48. Here the karyoplasm has entirely disappeared, leaving the nucleus empty and transparent except for the chromatin net, which has lost its connection with the nuclear membrane and contracted away from it toward the centre of the nucleus. All possible gradations are found between this stage and such a condition as is shown in Figure 53, where the entire chromatic structure is so densely packed that it appears to have fused into a single mass. As is shown in Figure 48, a massing of part of the reticulum may take place, while the remainder continues normal in aspect. The chromatin mass thus formed usually lies in contact with the nuclear membrane, but occupies no constant position in relation to the axis of the oval nucleus. Viewed from certain positions it is, of course, difficult to tell whether or not it is in contact with the membrane, though in some cases it is certain that it occupies a less peripheral position. In almost all cases a more or less complete resolution of the mass into its component strands is possible, and careful study has shown that these represent many very different stages. For example, the cell represented in Figure 48 is evidently in a stage immediately after the disappearance of the nucleolus; Figure 50 shows a contracted chromatin net, while Figures 54 and 55 show still later stages. This is not due, I think, to any progressive change of the nuclear structures during the stage of chromatic condensation, but rather, in all probability, to a nearly instantaneous condensation, which may occur at various periods — a conclusion supported by the observations of Lee ('97) on Helix. Indeed, we may safely say that in Gonionemus there is no stage in the prophase of the primary spermatocyte between the disappearance of the nucleolus and the dissolution of the nuclear membrane when it may not occur. This is of itself nearly conclusive evidence that the "pseudosynapsis" phase is not to be considered as representing a definite stage of development, and

there is in addition other evidence nearly as strong. Although these appearances are commonest in the primary spermatocytes, they are not entirely restricted to them, but occur occasionally in the "chromatin-segment" stage of spermatogonia as well, and likewise very commonly in the secondary spermatocytes, both before and after the disappearance of the nucleolus (Plate **4**, Figs. 74, 78, and 79); moreover they often appear in their most extreme type among the oögonia in the prophase (Plate **5**, Fig. 111). But whenever they occur in female germ cells it is always previous to the last oögonial division, where, of course, it can not be supposed that they have anything to do with synapsis, since in that mitosis no reduction in the number of the chromosomes occurs. Finally, perhaps the strongest evidence of all is the fact that, while in certain preparations and in certain individual medusae they occur in great numbers, in others they are comparatively rare; this latter condition was particularly the case in sections of a certain gonad in which all the normal prophases of the primary spermatocytes were exceedingly numerous. It is true, however, that they are present to a greater or less degree in every preparation, either section or isolation, examined.

In consideration of the foregoing evidence, I think we can not avoid the conclusion that, at least in Gonionemus murbachii, the "pseudosynapsis" phases are something entirely apart from the normal stages of the prophase of the first maturation division. There is one feature, and only one, possessed in common by all cells in Gonionemus which exhibit this phenomenon; that is the absence of karyoplasm — the "empty" condition of the nucleus. (By this I of course do not mean that it is actually empty, for no doubt it is occupied by transparent, probably inert, karyolymph). This, I think, gives us the key to the situation. Instead of being supported in a comparatively dense substance, as in the resting stages, the now much thickened chromatic reticulum is supported, if at all, only at its points of contact with the nuclear membrane, from which it easily breaks away. I think we must consider the segments of the spireme as being under tension, and thus particularly prone to shorten under slight influences. The fact that the massing is usually towards one side of the nucleus is readily explained by the reasonable assumption that the connection of the strands with the nuclear membrane is not likely to be equally strong on all sides, and that they would break away at the weakest points. Furthermore, when we recall how very common is the phenomenon of the shrinkage of nuclear structures under the action of reagents, we need seek no further, I think, for an explanation of their occurrence in the present case. It is, of course, only in the

living cell that this question can be definitely settled, and I trust that such a study of Gonionemus will not long be delayed.

Having, then, disposed in this way of the pseudosynapsis phases, I return to the consideration of the normal stages in the later prophase, leading to the formation of the individual chromosomes. To trace these in detail has been difficult, on account of the small size of the cells, yet the following phenomena are fairly well established. The last stage described (Fig. 47) was the "modified spireme," characterized by a thick, smooth, strongly staining chromatin net having comparatively few meshes. I have not been able to find any evidence here, any more than in the entoderm cells (page 298), or in the spermatogonia (page 306), that this net is ever metamorphosed into a continuous spireme thread. On the contrary, the next change appears to be the segmentation of the net, or rather of its chromatin component, into a considerable number of separate chromatin masses, which are roughly spherical, dense, and sharply outlined, but still remain connected to one another by delicate linin strands (Plate **4**, Fig. 56). This is a stage in which contraction very commonly occurs (Figs. 54, 55), so much so that I at first believed that the strands which contract to form the "pseudosynapsis" phase emerged therefrom segmented; this, too, is the course of events described by Guenther (:**04**) in Hydra viridis. The number of these masses, the chromomeres, is of course of great theoretic importance, and I have therefore made many counts. Absolute accuracy in these is perhaps impossible, certainly it is very difficult; but the counts varied always from 23 to 26 or 27, from which I infer that their actual number is probably twenty-four, and thus exactly equal to that of the chromosomes in the spermatogonia, and only half as great as that of the chromomeres in the same cells. Moreover, they are decidedly larger than the latter (compare Plate **2**, Fig. 23, with Plate **4**, Fig. 56). The nuclear membrane now breaks down, and the nuclear space becomes filled with cytoplasm, in which the chromatin structures lie more or less irregularly arranged, being still connected by the persistent achromatic strands, and less crowded than in the case of the spermatogonia. They also become more sharply outlined, denser, and stained more deeply (Figs. 57, 58). Enumerations of all favorable specimens gave, as before, twenty-four as the probable number of chromomeres. The formation of the chromosomes of the first maturation division takes place, as I believe, through the fusion of these chromomeres in pairs, just as is probably true of the spermatogonia, only here, since we are dealing with half as many chromomeres, only one half the somatic number of chro-

mosomes results. In its essentials the process, which is a rather difficult one to trace, is probably as follows. The chromomeres draw together in pairs (as in the cell represented in Figure 60), which finally fuse so completely that the component parts can no longer be distinguished. The cell shown in Figure 60 is particularly instructive, since in it six such pairs have already formed, while the remaining (twelve) chromomeres are still entirely separated from each other. In Figure 61 the process is practically completed, although the double nature of each of the chromosomes is still apparent, and even the connecting linin strands yet persist. Simultaneously, upon the fusion of the chromomeres, the twelve resultant chromosomes arrange themselves in the "equatorial plate" for the first maturation division. The chromosomes, to be regarded on account of their origin and reduced numbers as bivalent, are thus formed by the pairing of preëxisting chromatin structures, in exactly the same way that they are in the case of the spermatogonial division. In discussing the probable meaning of the present type of numerical or "pseudo" reduction of the chromosomes it is essential to keep this fact clearly in mind.

*Metaphase.* — As stated above, the chromosomes in the metaphase of the first maturation division (Fig. 61) are only half as numerous as the somatic chromosomes. The spindles at this phase are of two kinds, presenting in side view very different aspects, a fact which for a long time proved very puzzling (Figs. 64, 65); but careful study of many preparations has convinced me that the differences are apparent rather than real, and are due to external causes, not, as I at first supposed, to the occurrence of two types of mitosis. The apparent differences between the two are shown in Figures 64 and 65. The form represented in Figure 64 is by far the more common, and is no doubt to be regarded as the typical one. The chromosomes, here distinctly dumb-bell-shaped, are elongated in the direction of the spindle axis; they are apparently dividing, not by means of a longitudinal split, but by a gradual process of pulling apart. With careful focussing they can often be counted with comparative accuracy, even in side views of the spindle, and there is no doubt but that they are present in the reduced number, probably twelve. Such cells are very numerous, — in sections, always densely packed and usually occurring in groups of from five to twenty, amid spermatocytes of the same generation, but in different stages of the prophase. They lie near the margin of the gonad immediately beneath the peripheral layer of secondary spermatocytes, spermatids, and spermatozoa. The chromosomes are seldom well shown in polar views owing to their great

length. In division the thickened ends of the dumb-bells gradually draw apart (Figs. 62–64); they finally separate in the middle, although the tapering ends of the two daughter chromosomes often persist for a long time, as in the cell represented in Figure 64. As a rule, one or two of the chromosomes precede the others (Figs. 62, 63, 64). In some cases, however, division is more rapid and nearly simultaneous, the daughter chromosomes, here oval and sharply outlined, forming typical daughter plates. In the few instances in which it has been possible to count them there were apparently twelve in each daughter plate (Fig. 67). In any event, the chromosomes are distinctly larger than those of the spermatogonial divisions. Although this is the ordinary type, observed in about 175 cases, I have in perhaps fifteen or twenty instances noted spindles presenting the appearance shown in Figures 65 and 66. The splitting of the chromosomes, instead of being a gradual process, is here evidently rapid, so that there is no trace of the formation of "dumb-bells." The chromosomes are, on the other hand, rather irregularly arranged in the equatorial region of the cell, seldom or never forming a typical plate; accurate counting has been very difficult, but in every case there were considerably more than twelve. The migration of the chromosomes toward the poles is not simultaneous. It is very certain that the chromosomes in such a stage as is shown in Figure 66 have already undergone division: they show no trace of a double nature, and as in the type described above are larger than the spermatogonial chromosomes. Finally, the diameter of the spindle is considerably greater than in the more usual form, the maximum observed being 5 $\mu$; this, however, is of itself not a very important feature, since an unbroken gradation occurs in this respect.

As to the relation of this type of mitosis to the usual process of the first maturation division, the following evidence is of importance. In the first place, its comparative rarity (among several thousands of cells I have observed it at most not above twenty times) argues against its representing a determinate phase of spermatogenesis. There is also internal evidence for regarding it as merely a variation from the ordinary process. I have been able to find but a single line of development in the prophase, and the functional spermatids and spermatozoa are of but a single sort. The chromosomes in this, as in the more usual condition, are distinctly larger than in the spermatogonia. We cannot, I think, regard these spermatocytes as the parents of the giant spermatids, to be described later, for if they were, we should expect to find traces of a multiple nature, either in their size or in the number of centrosomes; but

this is not the case. Furthermore, there are certain gradations between the two. One such is represented in Figure 62, in which two of the chromosomes have separated before the others, the latter showing the elongate dumb-bell-like form.

In seeking the conditions which determine the occurrence of one or other of these two forms of spindles, we must, I think, turn to external causes. The rarer of the two is found on sections near the base of the gonad only, in the neighborhood of the mesogloea, occurring in isolated cells surrounded by spermatogonia; while the more typical spindles occur in groups in a more peripheral position, where primary and secondary spermatocytes, spermatids, and spermatozoa form a densely crowded layer. From this fact it seems not improbable that the apparent difference may be due either to the relative amount of pressure to which the cells are subjected, or to the readiness of their access to a food supply. It is interesting in this connection that similarly Downing (:05) found in Hydra the occurrence of two kinds of mitosis in the spermatogonia, a deviation from the common type being seen in cells which divide near the margin of the spermary, where the process more nearly resembles that of interstitial and ordinary ectoderm cells. The final result, however, was the same in both.

*Anaphase.* — As the chromosomes approach the poles they become, as in the last spermatogonial division, closely compacted together (Fig. 68); yet careful focusing always resolves the mass into separate component bodies. In the migration one chromosome often reaches the pole in advance of all the others (Fig. 62); this, however, is not invariably the case.

The achromatic figure of the first maturation division is of the same simple type as that seen in spermatogonia and in entoderm cells, its most interesting feature being, as there, the total absence of astral radiations. The spindle fibres are exceedingly delicate, and after the anaphase are no longer distinguishable as such, while the interzonal filaments are stout, stain deeply, and in the telophase bear a series of prominent Zwischenkörper, each of which is clearly a thickening of one of the filaments. There appears to be a single filament attached to each chromosome; consequently there are only one-half as many as in the preceding cell generation. The centrosome, a single minute granule without surrounding sphere, is first visible in the early metaphase, and cannot be traced with certainty after the breaking down of the spindle fibres in the anaphase.

Before proceeding to describe the second generation of spermatocytes,

we must pause to consider the most important question connected with the first maturation division; that is, whether it is a reducing or an equation division. It must be admitted that the internal evidence bearing on this point is but slight, and not very convincing. Since there is no apparent formation of tetrads, nor any trace of longitudinal splitting of the spireme strands, it is vain to turn for light on the sequence of the maturation divisions to the prophase, as McClung (:05) and Blackman (:05) have so successfully done in arthropods and Montgomery (:00) in Amphibia. The manner of cleavage can seldom be ascertained with certainty — as is now well understood — by studying the behavior of the chromosomes during the metaphase, since they are then so compacted that the planes of cleavage, if any exist during the late prophase, are wholly obliterated. This stage does, however, afford some little evidence, although it is not conclusive. Taking, in the first place, the events of the late prophase, we have seen a series of phenomena, which, except that we are here dealing with only one-half the somatic number of chromatin bodies, are exactly comparable to those seen in the spermatogonia, in which the division is, beyond any doubt, equational. The separation of the chromosomes, now probably of a viscid consistency, through a gradual elongation and drawing apart of their thickened ends, suggests a very firm coherence of their halves and argues against their separation along a recent plane of synapsis. I believe that the weight of the little internal evidence which exists is on the side of the view that the first maturation division in Gonionemus is longitudinal, and that its daughter chromosomes are to be regarded as dyads. Yet I must at the same time admit that in this animal, as in Lumbricus (Calkins, '95), it is impossible from the history of the chromatin structures, to state definitely which of the two maturation divisions results in reduction and which in equation. Hydra is so nearly related to Gonionemus that Downing's (:05) results on that form are here of especial interest; I come to the same conclusion as Downing, who bases his view on the evidence that the daughter chromosomes of this division contain the same number of chromomeres as their parents.

*Telophase.* — The telophase of the first maturation division presents no features of especial interest. The chromosomes are closely massed near the poles of the spindle (Fig. 68), but later separate again, whereupon it is seen that they have become connected with one another so as to form an irregular network (Fig. 69), exactly as occurs in the preceding mitosis. The new nuclear membrane is now formed and the remnants of both centrosome and spindle disappear. At the same time the chro-

matin nets become looser, so that the nuclei present the appearance shown in Figure 70. The components of a pair of daughter cells remain united to each other by the bridge of interzonal filaments for a long time, in some cases even after the reappearance of the nucleolus (Fig. 71); but this bridge gradually dwindles and finally breaks down. A very interesting feature of this stage is the occasional appearance on the cell margin of a rudimentary filament or "tail" at the point where the centrosome was located when last visible (Fig. 70). This phenomenon, important as indicating the close relationship between the centrosome and such fibrillar structures, recalls the discoveries of Moore ('95), and more especially those of Henneguy ('98) and Meves (:00), who found fibrillae actually attached to the centrosomes of the primary spermatocytes in certain *Lepidoptera*. Unfortunately, I have not been able to trace any actual connection between these filaments and the centrosome, since in the few cases where the filaments were observed the centrosome could not be detected. The chromatin net breaks up into its component granules, and the nucleus passes into the so-called resting condition. The formation of the nucleolus takes place, as in somatic and spermatogonial divisions, by the fusion of granules (Fig. 71) which, as indicated by their staining reactions, are composed of chromatin, and are therefore probably derived from the disintegration of the chromatin net.

### 4. *The Secondary Spermatocytes.*

The secondary spermatocyte in the "resting" stage (Plate **4**, Fig. 72) is very small, measuring only 5 $\mu$ in diameter, being thus only about one-fifth the bulk of its parent cell; but otherwise, it presents no important differences from the same phase of the previous generation. The spherical nucleus is filled with dense karyoplasm, through which ramifies the delicate reticulum, bearing irregular thickenings at its nodes. The nucleolus is a small spherical mass, selecting strongly basic dyes; it is probably of purely chromatic nature, as is indicated by the method of its formation. The body of the cell is roughly polyhedral, or, if not crowded, nearly spherical; the cytoplasm is finely reticulate and contains no archoplasmic structures, nor does it enclose any of the metaplasmic masses so common in the two earlier generations. The staining reactions are those typical of "resting" cells in general, — cytoplasm, karyoplasm, reticulum, and karyosomes selecting the acid constituent, and the nucleolus alone the basic dye, in the Auerbach mixture. There

is little or no growth during the "resting" stage. Since cells of this generation — which lie near the outer surface of the gonad — are found separately and only in small numbers, it is probable that this stage is of but short duration.

### 5. *The Second Maturation Division.*

The early prophase of the second maturation division presents the same general features as those with which we are already familiar in corresponding stages in both spermatogonia and primary spermatocytes. The reticulum thickens, becomes denser, and stains more strongly (Fig. 73); the net-knots increase in size, and at the same time the karyoplasm becomes less dense and finally disappears, leaving the nucleus in the "empty" condition already described in other cell generations. This result follows in the secondary spermatocytes earlier than in their parent generation, and the "pseudosynapsis" phases which, as already mentioned (page 315), occur in them in considerable numbers, are frequently seen at a stage previous to the disappearance of the nucleolus. They are also much more irregular, and more clearly present the aspect of artificial modifications of the nucleus than they do in the primary spermatocytes. Occasionally, as in the cell represented in Figure 79, they present the most typical "pseudosynapsis" appearance, but more frequently the contraction is limited to a portion of the reticulum, leaving the remainder unaltered (Figs. 74, 77); it may even include the nucleolus (Fig. 76), a condition never seen in the preceding generation. A series of these structures showing various modifications is represented in Figures 74 to 79. Taken by themselves they are of little or no interest, for they would be classed immediately by any student as artifacts, the results, perhaps, of imperfect fixation.

On account of the very small size of the cells and of the closely crowded condition of the chromatin in the later stages leading up to the metaphase, it has been impossible to follow the prophase in as great detail as in the previous cell generations. Unfortunately it is just those processes which are of greatest theoretic importance, — namely, the ones which result in the formation of the individual chromosomes, — that have proved most baffling. Yet certain features of the process can be established. These are the first visible changes in the resting nucleus: the reticulum thickens and stains more strongly, and the karyosomes become more prominent (Fig. 73), while the nucleolus separates into several distinct masses (Fig. 75), thus in its disintegration exactly re-

versing its mode of formation. At the same time, as already noted, the karyoplasm becomes less dense, and soon disappears, leaving the nucleus in the "empty" and transparent condition (Fig. 80). After the final disintegration of the nucleolus the net continues to thicken, and the karyosomes appear to fuse along the course of the strands, the result of these processes being the formation of a chromatin net composed of a comparatively small number of more or less separate segments. In the present generation this net is seldom or never so homogeneous and continuous as in corresponding stages in the primary spermatocytes, but the majority of cells show the condition clearly seen in Figure 80, where the segments are fully formed in only a portion of the nucleus, while in the remainder the threads and karyosomes still remain more or less separated. I am by no means certain that any further concentration of the chromatin in the net form is normally attained, nor have I been able to find any evidence, any more than in the preceding generations, that this net is ever metamorphosed into a continuous spireme thread. But, unfortunately, the number of cells examined at this stage was rather small, so that it has been less easy to trace the actual conditions than in the previous generation, in which they were exceedingly abundant (page 315). As far as staining reactions are concerned, the cells under consideration show the characters typical of prophases in general. When treated with the Auerbach mixture, the nucleolus is always stained a very clear green. Up to the time of the disappearance of the karyoplasm, the reticulum and the karyosomes select the red (acid) dye, but after that event they select the green (basic), so that their chemical reaction evidently undergoes a reversal from basic to acid.

That the chromosomes of the secondary spermatocytes always appear to be formed before the breaking down of the nuclear membrane, constitutes a very striking difference between the prophase of the second spermatocyte and that of other cell generations. Just prior to the dissolution of the membrane, the cells present the appearance shown in Figure 81. The chromosomes are dense, oval, and still connected with one another by continuous linin strands. They are present, as we might expect, in the reduced number, probably twelve. The membrane now breaks down, the nuclear area is filled with cytoplasm, and the chromosomes arrange themselves in the equator of the cell in a typical plate (Fig. 84). In polar views of this stage, which are rather common in sections, it is comparatively easy to count the chromosomes, and there is, I think, no doubt that the reduced number is twelve (Figs. 82, 83). The spindle is exceedingly small, the equatorial plate being not over

2.5 $\mu$ in diameter, and its length in the metaphase only about 6 $\mu$. As far as the achromatic figure is concerned, it shows no marked differences from those already described, except that the centrosome is larger (Fig. 85). This may be connected with the fact that it is fated to persist throughout the entire course of division and in the daughter spermatid, whereas in other cell generations it disappears in the telophase. The spindle, as in all adult tissue cells of Gonionemus, wholly lacks astral rays; the spindle fibres are delicate, and rapidly dwindle during the anaphase, but the interzonal filaments are stout, granular, deeply staining, and evidently entirely different in nature from the spindle fibres (Fig. 86).

In this, as in the first maturation division, there is but little internal evidence to show whether it is to be regarded as reducing or equational. The chromosomes in the present case divide rapidly (Fig. 85), never forming the elongated dumb-bell-like structures so characteristic of the former case (Fig. 64). Moreover, while it is impossible in the metaphase, to note any very decided difference between the size of the chromosomes of the two maturation divisions, in the anaphase it is at once apparent that in the second they are very much smaller than in the first (compare Fig. 68 with Fig. 85). These conditions suggest that we are dealing here with a reduction division. The evidence is, however, too incomplete for any very definite conclusion as to the succession of the maturation divisions in Gonionemus.

The later stages of division present no features of special interest. During the anaphase the chromosomes of the two daughter plates are so closely crowded that they appear to fuse (Fig. 86). But this is not actually the case, for they soon move apart once more. They have now lost their sharp contours and are connected, probably at their previous points of contact, into an irregular network (Fig. 87).

### 6. *The Metamorphosis of the Spermatid.*

We may consider the stage shown in Figure 88 as the first in the history of the spermatid. Constriction of the cell body has taken place, but the two daughter cells are still connected by a bridge of interzonal filaments, at the middle point of which is a series of deeply stained interzonal bodies. The most striking feature of this stage is the condition of the centrosome. It will be recalled that in the case of the somatic and spermatogonial mitoses previously described, this body could not be traced beyond the anaphase, and that it was always an exceedingly minute granule. In the second maturation division, however, it is much

more prominent (Fig. 84), and in the spermatid it still persists as a very definite deeply stained granule, lying at the focus of the remnants of the spindle fibres (polar radiations, as already mentioned, do not occur), and in contact with the cell membrane, much as Görich (:03', :04) has described for Sycandra. Moreover, I may state at once that there is no period during the metamorphosis of the spermatid when the existence of the centrosome cannot be demonstrated. Connected with the persistence of the centrosome and of the remnants of the polar portion of the spindle (Fig. 89) is the fact that the spermatid nucleus (Figs. 88, 89), in the early stages, never takes up the position close to the cell membrane which is characteristic of spermatogonial and spermatocyte nuclei in similar stages, but always lies more nearly central. The average total diameter of the spermatid is 3.5 $\mu$, that of its nucleus, 2 $\mu$. The connection of pairs of daughter cells by the interzonal bridge persists, as after the first maturation division, for a considerable period, during which this structure becomes more and more attenuated, and the cells move further and further apart. But that this separation is due to a mutual repulsion, as Blackman (:05, p. 61) suggests, I am by no means prepared to admit. Preparations in which the cells have been crushed show that the bridge is of truly fibrillar nature, for in such the individual fibres are often more or less clearly separated from one another (Fig. 88). Occasionally, even before the complete separation of the daughter cells, a short, delicate fibre can be detected, arising from the region of the centrosome (Fig. 88); this is, doubtless, the earliest stage in the formation of the tail of the spermatozoön.

The stage shown in Figure 90 may be considered the beginning of the actual metamorphosis. The interzonal bridge has now broken down, the nuclear membrane has reformed, and the chromatin has the form of a distinct, deeply stained network, the remainder of the nuclear area being occupied by rather granular karyoplasm. In different cells the condition of the network varies, and I have not been able to determine whether there is a definite number of segments. The cytoplasmic body of the cell is comparatively large, has a dense, finely reticulated appearance, stains feebly with iron haematoxylin, though strongly with the ordinary plasma dyes, and occasionally encloses darker masses or granules. From the fact that I have observed these in only a few cases I am inclined to believe that they are not archoplasmic structures. The remnants of the polar portion of the spindle, and of the interzonal bridge still persist. But the fibrous appearance so characteristic of both in the telophase is now hardly distinguishable in either; they now ap-

pear as homogeneous masses, distinguished from the surrounding cytoplasm by the fact that they stain more deeply, are distinctly outlined, and totally lack the reticulate structure of the latter.

Staining with iron haematoxylin, either alone or followed by a plasma dye, is unsatisfactory for the study of the archoplasm. The most successful stains for differentiating this substance are the safranin-gentian violet dye, and iron haematoxylin followed by the iodide-iodine solution mentioned on page 290.

The centrosome at this stage appears as a black granule without any trace of surrounding sphere, lying at the margin of the cell. The tail filament, now always present, seems to arise from the centrosome, and is about equal in length to the diameter of the cell (Fig. 90). Whether or not this filament actually grows out from the substance of the centrosome is best discussed later, in connection with the "giant" and "multiple" spermatids. From this point on, important modifications rapidly occur in nucleus, centrosome, archoplasm, and cytoplasmic cell body. The centrosome, confining our attention for the present to this structure, is in all earlier stages a single dense granule, lying at the margin of the cell, opposite the broad side of the oval nucleus. During the progress of metamorphosis it migrates around the margin of the cell until finally it comes to lie in the prolongation of the long axis of the nucleus. This migration, however, is not completed until much later, only its commencement being shown in Figures 90 and 91. At the same time it increases somewhat in size (Plate 5, Fig. 92), and a delicate, deeply staining filament can be detected extending from it inward along the axis of the cell toward the nucleus. As in the case of the axial filament of the tail, it is perhaps doubtful whether this actually grows out from the substance of the centrosome, or whether it represents a modification of cytoplasm metamorphosed under the influence of that organ. The centrosome now divides into two equal, minute, black granules, which may at first lie, as Görich (:03a, :04) found in Spongilla and Aurelia, side by side at the margin of the cell, but very soon they take up a position radial to the cell (Fig. 93). These two bodies are still closely in contact, and it is only by careful focusing that their double nature can be determined. The axial filament now extends from the proximal centrosome inward nearly to the nuclear membrane, and this centrosome now migrates inward along the filament, as is shown by comparing Figures 93 and 94; in Figure 94 it is seen to lie about halfway between the nucleus and the distal centrosome, with which it is connected by the filament. Eventually it reaches the nuclear membrane and for a long time can be

distinguished as a dark spherule closely apposed to the latter, as is shown in the cell reproduced in Figure 95. During this process the distal centrosome retains its original position and structure unmodified, but the tail filament grows until it is five or six times as long as the diameter of the body of the spermatid.

We now return to consider the fate of the remnants of the interzonal filaments, and the remnants of the polar portion of the spindle now represented by an indefinite mass of archoplasm (Fig. 90) lying near the centrosome, which is as yet undivided. The changes taking place synchronously in these two structures are not always absolutely the same; either one may outstrip the other. The remnants of the interzonal filaments no longer lie in the long axis of the cell, but at one side, nearer or farther from the centrosome as the migration of the latter has progressed to a greater or less degree. During the division of the centrosome and the migration of its parts, these remnants rapidly dwindle in size, until they are represented by only a small homogeneous globule or vesicle (Fig. 92), and finally disappear altogether. As I have already stated, there is no evidence that they are cast out of the cell, and I believe that the substance of which they consist is broken down and absorbed by the surrounding cytoplasm, though doubtless still existing in the form of minute granules, which may reassert themselves in the formation of the acrosome. It is also possible that a part of this material may join the persistent archoplasmic structure derived from the polar portion of the spindle, as is suggested by the increase in size and irregular behavior of the latter. The polar remnants, at the time of the disintegration of the remnants of the interzonal filaments consist of a single small, roughly spherical mass (Fig. 90); but shortly afterward — by the time the inner centrosome has reached the nuclear membrane in its migration — two such masses are to be seen, both distinctly larger than the original one; of these one lies on either side of the inward (centripetal) extension of the axial filament (Fig. 93). These two bodies, however, are not sharply outlined from the surrounding cytoplasm, and are not to be regarded as distinct structures surrounded by the latter, but rather as concentrations of archoplasm, which, on account of their chemical composition, appear dense and deeply stained. The two masses continue to increase somewhat in size, but retain their relative positions on either side of the axial filament throughout the later progress of the metamorphosis. At this early stage (shown in Figure 93) I have never been able to find more than two such bodies, although in the mature spermatozoön, as later described, three or even four may frequently be dis-

tinguished, a fact which seems to argue for their origin from more or less dissociated archoplasmic granules scattered through the cytoplasmic reticulum, rather than for their direct morphological descent from either centrosomal archoplasm, or spindle remnants.

The acrosome, or perforatorium, is first distinguishable in the stage represented in Figure 97, where it consists of a small globule lying in the cytoplasm closely apposed to the nucleus at the pole opposite the inner centrosome, and showing a typical archoplasmic staining reaction. It has, unfortunately, been impossible to follow in detail the stages in its development, but it may not be out of place to consider its probable origin. As we have already seen, the remnants of the interzonal filaments at the time of the breaking down of the interzonal bridge lie in contact with the nucleus at the pole opposite the centrosome, and at first sight it might seem that they are metamorphosed directly into the acrosome. But, as we have also seen, they come to lie in a very different position, and then dwindle and finally disappear before the appearance of the acrosome, many cells (as, *e. g.*, that shown in Figure 95) possessing neither the one nor the other. The evidence, then, is wholly against the direct derivation of the acrosome from the interzonal remnants. Yet the evidence afforded by staining reactions — and I think that in the study of the spermatid these reactions are fairly reliable, more especially that to iodine — is to the effect that the acrosome is chemically identical with the two archoplasmic masses just mentioned as lying one on either side of the axial filament. Since there is no evidence in favor of the view that any other structure — nuclear substance, centrosome, etc. — takes part in the formation of the acrosome, I believe myself justified in saying that the acrosome is of archoplasmic origin. In the earliest view of it that I have seen it is a sharply outlined, deeply staining, homogeneous globule lying against the nuclear membrane (Fig. 97), and it persists in this position without any visible changes in structure or outline.

While the foregoing modifications have been taking place in the centrosome and in the archoplasmic structures, the nucleus has also undergone profound changes. In early stages, previous to the division of the centrosome, the chromatic substance is condensed into an irregular network, while the remainder of the nuclear area is filled with dense karyoplasm (Figs. 91, 92). The latter, however, shortly disappears, leaving the space apparently empty except for the chromatic net (Fig. 90). The nucleus itself is very small, the diameter being only 2 $\mu$, but during the migration of the proximal centrosome it increases considerably

in size, reaching its maximum in the stage shown in Figure 93, where its bulk is more than doubled. The chromatic net, at the same time, becomes much looser, lying close against the sharply defined nuclear membrane, leaving the central portion of the nucleus unoccupied. Condensation appearances, so characteristic of the spermatocytes, do not occur in the spermatids, probably owing to their small size. After attaining its maximum size (2.5 $\mu$) the nucleus diminishes once more. The chromatin becomes condensed in certain areas, losing its definite net-like arrangement, and frequently exhibits the appearance shown in Figure 96, where it forms two distinct masses near the poles, connected by an axial rod. With further decrease in the size of the nucleus, the chromatin becomes diffused evenly throughout it, so that the entire nucleus takes a dark stain. The basal chromatic mass appears, however, to persist, although of this I cannot be certain; it is probable that we must interpret as such the deeply stained plate which forms the base of the conical head in the adult spermatozoön (Fig. 102). At its maximum size the spermatid nucleus attains a diameter of 2.5 $\mu$, but diminishes to about 2 $\mu$. The chromatin, from the time when it becomes diffused throughout the nucleus, undergoes an important change in its staining reaction, for when treated with the gentian-safranin mixture, so useful in the study of spermatid metamorphosis, it takes the safranin dye as strongly as it previously did the gentian. This reaction is very helpful, since it strongly emphasizes the line of separation between the nucleus on the one hand and the archoplasmic structures and the acrosome on the other, both of the latter staining blue. The nucleus now becomes flattened on the side toward the tail filament, where it is bounded by a more deeply staining chromatic plate, and gradually changes as a whole from a spheroidal to a broadly conical form (Fig. 99). Seen in polar view it is circular (Fig. 100) and surrounded by a layer of cytoplasm. Meanwhile the proximal centrosome becomes more and more intimately connected with the nuclear substance, until finally it can no longer be demonstrated as a separate body, although represented by a minute prominence long visible at the point of fusion (Fig. 101). There is, however, in Gonionemus no direct evidence that this centrosome actually penetrates into the nucleus, I believe, therefore, that it merely becomes flattened against the basal portion of the nucleus, being obscured from view by the deeply staining property of the latter.

While these changes have been taking place, further modifications occur in the archoplasmic organ. This, when the nucleus was largest, consisted of two small masses at the base of the nucleus, which, with

the migration of the proximal centrosome, come to lie on either side of the axial filament. While the nucleus decreases in size these steadily increase in bulk until they occupy nearly the whole space between its posterior margin and the cell boundary, as is seen in the cells represented in Figures 99, 101. Such a cell seen in edge view in a direction perpendicular to that of the last mentioned figures, shows the appearance represented in Figure 98, where the two archoplasmic masses lie one above the other, the axial filament clearly passing between the two. With the assumption of the conical form by the nucleus, the growth of these archoplasmic bodies continues until finally they become closely apposed or even fused to each other, thus forming a more or less rectangular mass in contact with the base of the nucleus. But this mass still plainly consists of two deep-staining centres (Fig. 101); these, however, are now no longer morphologically distinct, as is shown by the fact that in crushed specimens, or in preparations macerated by the Hertwig method, they adhere to each other and do not separate as they do in earlier stages after similar treatment.

During the progress of metamorphosis the cytoplasmic cell body, as well as its various organs, undergoes a series of changes. In the early spermatid, at the time of its final separation from its sister cell, the cell body is comparatively large, its diameter being 3.5 $\mu$, and the cytoplasm is dense, finely reticulate, and throughout of uniform structure, enclosing no vacuoles or metaplasmic masses. This condition persists during the growth period of the nucleus and the migration of the proximal centrosome; but at the time when the change in the form of the nucleus commences, small vacuoles appear in the neighborhood of the nuclear membrane; these become more or less confluent, and soon form from one to three large ones. After this has taken place, a polar view of the cell shows the appearance represented in Figure 100, where the circular nucleus (seen of course in optical section) lies in a clear area, which is bounded by a shell of finely reticulate cytoplasm. The commencement of the same process is shown in side view in Figure 101, where, at the left of the nucleus there is a single large vacuole, while on the opposite side the cytoplasm still remains unmodified. This change progresses until finally the nucleus comes to lie free in a clear space, surrounded by only a thin shell of cytoplasm, a condition very similar to that figured by Downing (:05) in the case of the spermatid of Hydra fusca; but in Gonionemus the long axis of the nucleus continues to coincide with the axis of the cell, and never assumes the various aberrant forms described for Hydra. In later stages the cytoplasmic shell cannot be detected.

But I have been unable to determine whether this disappearance is the result of disintegration, or whether the cell membrane unites with the nucleus. The spermatozoön now appears to be mature, and by teasing out fresh tissue is seen to swim actively by means of the tail.

For the sake of comparison with the spermatozoön of other coelenterates it may be well to describe its structure in the finished condition, which is shown in Figure 102. The head measures 2.5 $\mu$ in length, the middle piece 1 $\mu$, and the tail at least 15 $\mu$, but I have not been able to determine the extreme length of the latter. The head and middle piece together have a broadly conical outline when seen from the side, but are circular in polar view. The nucleus is conical with a nearly flat base and slightly truncate tip; as a whole it stains homogeneously and deeply, exhibiting no distinct chromatic structures, except a dense, more deeply staining plate at its base (Fig. 102), which is, perhaps, derived from the basal chromatin mass of the stage shown in Figure 96. Closely in contact with the truncate apex of the nucleus is the acrosome, a spherical or oval body which exhibits the characteristic archoplasmic staining reaction. A well-marked middle piece can now be distinguished, the axis of which is the axial filament connecting the distal centrosome with the base of the nucleus, the sheath or mantle consisting of the archoplasmic bodies. Upon the disappearance of the cytoplasmic mantle, these bodies are seen to be spherical masses of considerable size, usually two in number. In many cases, however, I have clearly distinguished three or even four, so that we have in Gonionemus a condition comparable to that described by Retzius (:05) in Tubularia, Clava, Sertularia, Halecium, and certain actinians and lamellibranchs, where he finds that the number of archoplasmic masses in the middle piece of the spermatozoön is variable. The only persisting trace of cytoplasm is a very thin layer covering the base of the middle piece; at the margin of this the distal centrosome can be distinguished in favorable cases as a minute dark granule. The portion of the axial filament between the two centrosomes is usually entirely masked by the archoplasmic bodies, though occasionally, especially in maceration preparations, it is visible; but the proximal centrosome can no longer be detected. The question whether it still persists, or has actually disintegrated, can not be definitely answered. The tail consists of a single delicate fibre. It seems to arise from the distal centrosome, through which it is directly continuous with the axial filament of the middle piece; this is an argument for believing that the latter is a true fibre and not merely a modified "track" in the cytoplasm or archoplasm, as has sometimes been sug-

gested. I have not been able to trace, at any stage, a sheath of either cytoplasmic or archoplasmic material surrounding the tail.

### 7. *Giant and Multiple Spermatids and Spermatozoa.*

While the vast majority of spermatozoa exhibit the structural conditions just described, I have found a considerable number of abnormalities, falling into two well-marked classes, which may be termed respectively "giant" and "multiple." I have been able to trace some of the stages in the metamorphosis of the first of these, finding that they agree in their general features with those of normal cells, and strongly recall the conditions found by Paulmier ('99) in the "giant" spermatids of Anasa. Such spermatids are first distinguishable after the telophase of the second maturation division, and probably result, as Henking ('91, page 718) and Paulmier ('99, page 254) suggest, from the non-completion of the first or second spermatocyte division. In early stages of metamorphosis their most striking peculiarity, the possession of several tails, is not yet developed; for this reason, as well as because they are present in very small numbers, they might easily be taken for the larger cells of an earlier generation. But when they reach the stage shown in Figure 103 (Plate 5) their true nature is at once apparent from the multiple condition of tails and centrosomes. They are either double, triple, or even quadruple, as is shown by the size of the nucleus, and by the number of tail filaments and centrosomes. The earliest stages detected, one a double, the other a triple individual, are shown in Figures 103 and 104. In the former the nucleus, which is evidently in its period of greatest size, is 3.5 $\mu$ in diameter and presents an appearance similar to that of the normal spermatid. The whole cell is 6.5 $\mu$ in diameter, and therefore about twice as large as the normal spermatid. The remnants of the interzonal filaments are still visible as a conical mass in contact with, and near one pole of, the slightly oval nucleus. The principal archoplasmic organ — probably derived here, as in normal metamorphosis, from the remnants of the polar portion of the spindle — consists of a single large body, lying near the opposite pole of the nucleus, and containing three or four deeply staining regions. There are two centrosomes, as yet undivided, lying about 30° apart, at the periphery of the cell, one on either side of the archoplasmic mass. There are also two distinct tails, each a simple filament arising from one of the centrosomes, while extending inward from each of the centrosomes is a short axial filament, not yet, however, reaching to the nucleus.

The triple specimen (Fig. 104) is in a slightly more advanced stage. The nucleus, in about the same phase as the one last described, is in this case nearly spherical. The remnants of the interzonal filaments have entirely disappeared, but no acrosome is yet visible. The archoplasmic organ, lying in the same position with reference to nucleus and centrosomes as in the double cell, is here clearly differentiated into three deeply staining centres (Fig. 104), which are not, however, separate masses. There are three centrosomes, minute granules lying at the periphery of the cell, about 10° apart, one opposite each of the archoplasmic centres. From each centrosome arises a distinct tail filament, all these being more or less twisted together, as is commonly the case. I have not been able to trace any forward extension of the axial filament in connection with the two lateral centrosomes, but from the middle one such a structure is clearly visible, extending inward to the nuclear membrane. At its point of junction with the membrane there is a deeply staining knob, no doubt to be regarded as having resulted from the division of the middle centrosome into proximal and distal parts. Apparently, however, the two lateral centrosomes have not yet divided. The nucleus is considerably larger than in the double spermatid (Fig. 103), measuring 4 $\mu$ in diameter.

The giant spermatids, whether double, triple, or quadruple, agree with normal cells in the nature of their later metamorphoses, and differ from one another only in size and the number of their tail filaments and centrosomes. The nucleus becomes homogeneous, stains deeply, and assumes the typical conical form; a spherical acrosome of archoplasmic nature develops at the slightly truncate apex of the nucleus, the cytoplasm, previously finely reticulate, becomes vacuolate in the neighborhood of the nucleus, which finally comes to lie in a clear area (Figs. 105 and 106). The archoplasmic mass increases in density and bulk, and shows the separation into well-marked spherical masses characteristic of the normal spermatozoön (compare Fig. 102 with Fig. 106). There now occur other changes which point strongly toward degeneration as the ultimate fate of these giant spermatozoa. Clear vacuoles (Fig. 106) appear in the dark substance of the nucleus; the cytoplasm is often ragged and torn; the tail filaments appear to fuse (Fig. 105), and then dwindle (Fig. 106); and the centrosomes become less sharply defined, as though being gradually diffused. Finally, in many cases all organs of the cell — cytoplasm, middle piece, etc. — are broken down, the nucleus and acrosome alone being recognizable. In certain preparations many of these isolated nuclei are to be seen. There is, then,

good reason to believe that such cells never attain functional maturity, but that they sooner or later degenerate. It is interesting to note in this connection that some individuals appear to produce them in comparative abundance, while others do so but seldom, if at all.

In addition to the giant spermatozoa just described, there are to be seen in all preparations of the adult spermary of Gonionemus a considerable number of multiple spermatozoa, of which two specimens are represented on Plate 5 (Figs. 107, 108). These exhibit all possible conditions of partial separation, but agree in this that the nuclei have divided and separated more or less completely, while the cell bodies have failed to divide, thus differing from the giant spermatid, in which both nucleus and cell body have failed to divide. Combinations of the two conditions may, however, occur, as is shown by the specimen represented in Figure 108.

A typical example is shown in Figure 107. Here there is a single cell body enclosing two perfectly formed nuclei, one of which exhibits at its apex a typical acrosome. Although the nuclei are apparently normal in structure, they invariably exhibit a much more elongate form than is the case with normal spermatozoa, a condition due in all probability to their being crowded together within a single cell body. Two distal centrosomes are visible at the periphery of the cell, and extending inward from each of them is a well-defined axial filament, but a proximal centrosome is to be seen in the case only of the nucleus without an acrosome. From each of the distal centrosomes there arises a tail filament of entirely normal appearance and length. The example shown in Figure 108 is an interesting modification of this condition, because while the smaller of the two nuclei presents the appearance just described, the other, which is nearly twice as large, is double. That it is really double is shown not only by its large size, but also by the existence of two tails and distal centrosomes. In all the multiple spermatozoa the archoplasmic organ is morphologically a single structure, although it may exhibit two or more staining centres, — a fact of some significance in connection with the question of the origin of the tail filament.

### C. Oögenesis.

In the oögenesis of Gonionemus I have been able to trace the history of the germ nuclei from the prophase of the last oögonial division until the germinative vesicle comes to lie at the surface of the nearly ripe

oöcyte. From this point on to the close of the second maturation division a gap, due to lack of material, still exists in my observations. Although the portion of the oögenetic process here described is less important in connection with the questions of reduction than are the later stages, yet, it does cover certain phases of importance from a more general cytologic standpoint. It is of theoretic interest to establish conclusively in this primitive class, whether the chromosomes retain their individuality from the close of the last oögonial mitosis throughout the growth period of the oöcyte, as Häcker ('92b) found to be the case in some copepods, and Rückert ('92) in selachians; or whether, as is no doubt the case in the majority of Metazoa, the nuclear contents enter during this period into a resting condition with diffuse chromatin.

I have not studied the younger stages in the formation of the gonad in Gonionemus, and so have not been able to attack the question of the origin of the female germ cells in that genus, but from conditions easily traced in Olindias, a closely allied form, I believe that they are of ectodermic origin and originate in the region of the gonad, not migrating from elsewhere, as is so commonly the case among the Anthomedusae and Leptomedusae. In all the many sections of radial canals examined, I have never found any evidence of the existence of any such migratory cells in Gonionemus.

The ovary (Fig. 1) is, in its general features, similar to the spermary, presenting the appearance well known and often described among those hydromedusae whose sexual products are borne along the radial canals. A section invariably shows a number of nearly ripe eggs, others smaller, and, packed between these, often basally, small groups of oögonia and young oöcytes. Since this structure is typical, it is unnecessary to describe it more fully here, and we may proceed at once to consider

### 1. *The Oögonia.*

In sections of the gonad these cells (Fig. 1, *o'go*) are seen in small groups of from five to ten, in the spaces between the ripening oöcytes, frequently in contact with the mesogloea, but occasionally in a more peripheral position; indeed, the only part of the gonad where they do not regularly occur is at its surface, which is covered by a layer of ectoderm cells easily distinguished by their exceedingly small nuclei. The oögonia are not very numerous, and appear to divide but rarely, or at least at long intervals; so that it has proved much more difficult to trace the stages in mitosis than is the case with the spermatogonia. It

is, however, of theoretic importance, as will appear later, to determine definitely the relationships of the few division stages which do occur.

The history of the chromatin exhibits no important differences from that of the ordinary somatic cells, although from the small size and general arrangement of the chromosomes, it is in the later stages easily distinguished from that of the latter.

The resting oögonia (Plate 5, Fig. 109) are similar in appearance to the spermatogonia. The nucleus, which measures about 6 $\mu$ in diameter, contains as a rule a single large nucleolus, from which there radiate throughout the nucleus the threads of a delicate linin reticulum with prominent karyosomes at its nodes. The nucleolus itself shows a deeply staining external shell enclosing a paler central mass, an appearance with which we are familiar in so many other cell generations in Gonionemus. It is no doubt of composite nature. The remainder of the nuclear space, as in other resting nuclei, is filled with rather dense and distinctly granular karyoplasm. During the "resting period" which precedes the last mitosis, the oögonia undergo considerable growth, and previous to the commencement of the prophase the cytoplasmic body of the cell (Fig. 109) is of comparatively large size and frequently contains one or more of the deeply staining metaplasmic masses so common in the spermatogonia. But, as in the latter case, these may often be absent. No archoplasmic structures are present, nor can the presence of a centrosome be certainly demonstrated. At this time the staining reactions of the oögonia are those characteristic of resting cells in general, the cytoplasm, karyoplasm, and karyosomes showing a basic, the nucleolar shell alone an undoubted acid reaction.

The general course of the prophase changes simulates very closely that of the entoderm cells. The nuclear net thickens and stains more deeply, while the karyosomes at its nodes grow more prominent (Fig. 110). The later changes are less easy to study than is the case in spermatogenesis, for the reason that it is necessary to trace them chiefly or entirely in sections,—a method, much less satisfactory when dealing with such structures as chromatin segments, than the isolation method, which allows of the examination of entire nuclei. The reason for this necessity is twofold. In the first place, dividing oögonia are so rare that it is difficult to find them at all in isolation preparations, and in the second place, their nuclei so closely resemble those of the entoderm cells in size and general appearance that there is great danger of confusing the one with the other.

With the increased density of the nuclear net the nucleus loses its

spherical outline and becomes oval (Fig. 110), a fact which it is necessary to bear in mind when comparing the sizes of cells viewed in different directions (compare Figs. 110 and 112); at the same time, as in other cell generations, the karyoplasm becomes less and less dense, and finally (Fig. 112) disappears altogether, leaving the nucleus in the apparently empty condition with which we are familiar in other mitoses in Gonionemus.

Hand in hand with these changes the net continues to thicken, while the karyosomes increase in size and numbers, many of them now lying (Fig. 110) along the courses of the strands, instead of only at their nodes. This process continues, and the chromatin masses become more and more crowded until finally (Figs. 113, 114) the net consists of a considerable number of thickened beaded segments connected with the nucleolus and with one another by linin strands. This is essentially the condition presented by the entoderm cells at a corresponding stage. The nucleolar shell now separates into several distinct chromatin masses (Fig. 113), which seem to migrate outward along the linin strands at whose points of origin they were situated, and the denuded plasmosome is now visible lying between them (Fig. 113). This latter structure must shortly break down or be absorbed, for it is absent in all later stages.

At various periods in the prophase, often even before the disintegration of the nucleolus, contraction phases similar to those already described among the male germ cells (page 314) are found, and here they usually take the form of extreme condensation, as is shown in the cell represented in Figure 111. The occurrence of such appearances has already been discussed at length (see page 315). In the present case the chromosomes appear, as would be expected, in the full somatic number; they therefore cannot be connected with any process of synapsis, but are purely the result of the action of reagents. The later prophase is very similar to that of the entoderm cells. After the final disappearance of the plasmosome the cells present the appearance shown in Figure 114. The entire chromatic substance of the nucleus is now condensed into a considerable number of "beaded segments," which, being still connected by achromatic strands, preserve the net-like condition. I have never seen any indications that they become metamorphosed into a single continuous spireme thread. I believe, however, that we must regard this stage as homologous with the segmented spireme, since there appears to be no further segmentation of the chromatic strands. The nuclear membrane now breaks down, and the nuclear space is invaded

by cytoplasm, the result of this being, as in the mitoses already described, a crowding or contraction of the chromatic structures (Fig. 115) which makes it difficult to trace in detail the formation of the chromosomes from the chromatic strands. There is, as already stated, no trace of the occurrence of chromomeres, as is the case in the spermatogonia and primary spermatocytes, but here, as in the entoderm cells, the chromosomes that appear in the metaphase are probably formed by a simple contraction and condensation of the segments. The cell represented in Figure 115 shows stages in this process, which as a rule does not take place synchronously throughout the whole of the nucleus. The chromosomes are still connected with one another by linin strands, which, for a brief period after the disappearance of the membrane, seem stouter and stain more deeply than before (compare Fig. 114 with Fig. 115); but they disappear shortly after, so that by the time the chromosomes come to lie in the equatorial plate no trace of the achromatic strands is to be seen.

The metaphase of the oögonial mitosis so closely resembles that of the spermatogonial as to be practically indistinguishable from it. The chromosomes form a typical plate, and both spindle and centrosomes can now be seen for the first time. The achromatic figure (Fig. 118) is exceedingly simple; astral radiations are entirely lacking; and it in every way resembles those of both somatic cells and male germ cells. The chromosomes, which in the metaphase are distinctly dumb-bell-shaped and very small, lie with their long axes parallel to the plane of cell division (Figs. 116–118), and are generally well separated, so that it is rather easy to count them. Unfortunately, the apparent number increases with the progress of the splitting, so that to estimate the typical number care must be taken to choose only the very earliest steps in the metaphase. From the few of this stage which I have been able to study I have no doubt that the number is the same as in somatic cells, in all probability twenty-four.

The splitting of the dumb-bell-shaped rods is longitudinal, and begins at one end; so that when viewed from the pole of the spindle they form V-shaped figures (Fig. 116), which later become separated into daughter chromosomes, each having the same constricted form. The daughter pairs seldom lie directly one above the other, and it is therefore often possible in polar views of the late metaphase to count nearly twice the somatic number, as is shown in the cell represented in Figure 116, in which thirty-six are visible. The form of the chromosomes seems here to have no special significance; at any rate, it is not connected with any

pairing process during the prophase. When the spindle is seen sidewise (Fig. 118) the chromosomes appear as small circular masses; and the spindle as a whole is comparatively broad and short. As a rule, the splitting takes place in all chromosomes before the migration to the pole commences, and this migration being nearly simultaneous, daughter plates are formed (Fig. 119). But unfortunately I have not been able to count the number of the chromosomes in any of these. During the migration the chromosomes usually preserve their original orientation (Fig. 119), but occasionally they turn so as to lie with their long axes nearly parallel with the spindle fibres. The later anaphase and the telophase present no features of special importance. The chromosomes, as they approach the poles, become closely crowded together, though never completely fused, and when they once more separate are seen to be connected together in an irregular network, perhaps through confluence at their previous points of contact, just as takes place in other cell generations. With the migration of the daughter chromosomes interzonal filaments are formed, which, as is usual in this animal, are much stouter and of more clearly fibrous nature than the spindle fibres. At the end of the anaphase the latter break down; the centrosomes disappear — though they may of course persist undetected — and with the constriction of the cell the interzonal bridge also disappears, so that the two daughter cells become entirely separate, instead of remaining connected, as so commonly occurs in the maturation divisions of the male germ cells. This mitosis presents, as such, no especial points of interest, except for the very striking resemblance of its metaphase and anaphase to those of the spermatogonia, and the corresponding difference from the entoderm cells (compare Figs. 116, 118, 119, with Figs. 30, 31, 32). The feature important to be borne in mind is that all the mitoses occurring in the female gonad are certainly of this one type, and, as certainly, all belong to the same cell generation, the oögonia. It was chiefly to establish definitely whether or not this was the case that I followed through the mitotic changes in detail.

At the close of the oögonial mitosis the nucleus, after a brief telophase, in which the nuclear membrane re-forms and its contents arrange themselves in a loose net, passes into the so-called "resting" condition, as is the case after all the other cell divisions studied. The nucleolus reappears; the nuclear space becomes filled with dense karyoplasm, and the chromosomes entirely break down and pass into the diffuse condition so commonly described.

It is thus certain that in Gonionemus after the last oögonial division

the chromosomes do not persist as such; they do not retain their individuality during the later growth period of the oöcyte. Nor is there during the telophase any evidence of the occurrence of synapsis, so that we must, no doubt, look for the occurrence of this process at a much later stage.

### 2. *The Oöcytes.*

At the close of the last oögonial division the daughter cells, now oöcytes, enter upon a typical "resting" stage, during which they undergo very considerable growth. This, however, is not the commencement of their final growth period, for very significant nuclear changes take place prior to that period. An oöcyte in this stage is represented in Plate 5, Figure 120. The nucleus exhibits the usual reticulate condition already described in corresponding phases both of somatic and of male germ cells (pages 296, 302), there being, as a rule, a single very large nucleolus, from which delicate linin strands radiate outward through the rather dense karyoplasm. The nucleolus itself shows a deeply staining external shell enclosing a more transparent central mass. The cytoplasm is finely reticulate and occasionally encloses metaplasmic masses, but no trace whatever of any archoplasmic structures, such as Nebenkern, sphere, or centrosome, can be detected. When treated with the Auerbach mixture, nuclear membrane, karyoplasm, reticulum, and karyosomes select the acid, while the entire nucleolus selects the basic dyes. The cell grows until the nucleus attains a diameter of about 8.5 $\mu$, when there occur nuclear changes which suggest the prophase of an ordinary mitosis. The occurrence of such appearances is remarkable, for, as we shall see (page 345), it is almost certain that they do not lead to any cell division whatever; therefore they must be considered in detail.

*Pseudoprophase.* — The first change in the nucleus is, as in ordinary mitosis, a thickening of the reticulum (Fig. 121), which now, more conspicuously than before, radiates from the nucleolus, while the karyosomes increase in both size and staining capacity. At the same time the karyoplasm becomes gradually less and less dense until at last it disappears altogether, leaving the nucleus in the typical "empty" condition. Many karyosomes now come to lie along the course of the threads of the net, instead of only at its nodes, as was previously the case; and at the same time the nucleolus suffers a decrease in bulk (compare Fig. 120 with Fig. 122). As the karyosomes, lying along the strands, increase in number, they become more or less crowded together, thus forming rather definite chromatin segments having a "beaded" appearance. Fig-

ure 122 shows three or four such segments, while the remainder of the reticulum still preserves the more primitive condition. Thus the condition of the nucleus as a whole is now similar to that of somatic cells and oögonia at a corresponding stage. As this process of condensation of the separate chromatic masses, together with the gradual disintegration of the nucleolar shell continues, the segments of this kind increase in number, until at last a stage is reached where they constitute all the strands of the net (Fig. 123), being quite sharply separated from one another, although still connected by persisting linin strands. It is now clearly seen that the chromatin shell of the nucleolus (Fig. 123) has separated into a variable number of separate chromatin masses, which probably migrate outward along the linin strands. The final result of the process is that all the chromatin is collected into the "beaded" segments (Figs. 125–127), leaving the nucleus otherwise entirely empty except for the connecting achromatic threads and the central portion of the nucleolus, a sharply outlined, strongly staining spherical structure, which persists after the disintegration of the nucleolar shell.

While this series of changes has been taking place, the cytoplasmic body of the cell has dwindled, and during the remainder of this phase of nuclear activity is very distinctly smaller than in the preceding "resting" stage, a condition similar to that already described in the case of the first generation of spermatocytes (see page 314), and one which points to the great metabolic activity of the nuclear structures.

Since it is at a corresponding phase in the maturation division of the male germ cells that contraction phases so commonly occur, it is of interest to observe that here, too, such appearances are occasionally seen, but only rarely, and then never in an extreme condition. In the later "segment stage" they are never seen, probably because of the stoutness of the chromatic segments themselves.

The cells now present a very characteristic appearance (Figs. 125–127), and owing to their comparatively large size, allow on isolated specimens a very accurate mapping of their nuclear contents. Fortunately, with material preserved in Flemming's fluid, the female gonads lend themselves quite as readily to the making of isolation preparations as do the male gonads, so that I have been able to study a considerable number (over one hundred) of entire nuclei at this stage. From sections the nuclear conditions are very much less easily resolved, since the section plane frequently cuts the chromatic segments, and reconstruction of these from adjacent sections is accompanied by much difficulty and

chance of error. A typical specimen, and one which shows the relations of the nuclear structures perhaps as clearly as any, is represented in Figure 127. There are in this case twelve or thirteen beaded segments very loosely connected with one another and with the nucleolus by exceedingly delicate linin threads. The number of "beads" in each segment varies from four or five to eight or nine, and similar variation was seen in all the cells examined. The segments themselves, which are very stout and sharply outlined, vary both in length and form, being either nearly straight, or U-shaped, or variously bent and twisted. Their number seems also to be variable, there being as a rule from twelve to fifteen; in many cases, however, several may be so intimately connected as to make their resolution impossible. I believe, that owing to this variability, they cannot be homologized with chromosomes, though unfortunately on this point, which is one of great theoretic importance, their later history throws no light. Their frequent exhibition of U-, V-, and Y-shaped forms (Figs. 126, 127) at first suggests the possibility that this may be a stage of synapsis; but the internal evidence on this point is too scanty to be convincing, and the later stages of the oöcyte are such as probably to forbid our accepting this view. I, therefore, believe that, if we are to homologize this stage with any part of the usual prophase of mitosis, it must be with the spireme.

The central part of the nucleolus (Figs. 125–127) is, in addition to the chromatic structures, a constant feature of the nucleus. This body, which is spherical, frequently vacuolated, and very much smaller than the compound nucleolus present at the commencement of this apparent prophase, appears to persist from the telophase of the last oögonial division till the time when the ripe oöcytes are released. Finally, at no time, at least up to this point, can any trace of centrosome, sphere, or other archoplasmic structure be detected.

The steps in the process from the resting stage to the formation of the "segment" or "modified spireme" stage just described are traced very easily, but the later changes are much more difficult to follow, and still remain somewhat obscure. We should naturally expect the chromatin-segment stage to be followed by division; but, although I have searched several hundred sections, I have found no evidence whatever that this is the case. It seems, on the contrary, almost certain that this stage is the culmination, so to speak, of this phase of nuclear activity, and that it is followed by regressive changes resulting eventually in the assumption once more by the nucleus of the so-called resting condition. Since this conclusion — that this apparent prophase is not followed by

any mitosis, but leads once more through regressive changes to a "resting condition" — is one of great theoretic interest, I will summarize the lines of argument which make it, to my mind, the only reasonable one. Negative evidence, it is true, is never altogether satisfactory; the fact that no mitotic stages are found being of course not conclusive that none occur; there is, however, certain circumstantial evidence of this which nearly amounts to proof.

In the first place, in every mitosis in Gonionemus, although early prophases may be rather rare, as in the somatic cells and in oögonia, mitotic figures are from their conspicuous nature always easily found, furthermore, the late prophases are quite as abundant as the earlier ones. For the sake of comparing the abundance of the "prophases" of the oöcyte with that of other generations, I have counted them, and have so far noted three hundred cells in stages between those represented in Figures 122 and 127. They are thus very much more numerous than corresponding phases in either oögonia or somatic cells. Yet I have not found a single case where the plasmosome is not clearly visible, where the nuclear membrane had broken down, or where, in short, the course of events had progressed any further than the typical "segment stage" represented in Figures 125, 126, 127. The evidence of the persistence of the nucleolus I consider very important, since in all mitoses stages immediately after its disappearance are quite as numerous as those just prior to that event. Still further, although we might perhaps assume that the lack of mitotic figures was due to the great rapidity of this mitosis, we can hardly suppose this to be true of the whole series of important changes leading from the spireme stage to the appearance of chromosomes in the metaphase. It has been suggested that we might consider the mitosis, which I have already described as belonging to the last oögonial division, as being in reality the outcome of the conditions now under consideration. But this is, I think, conclusively negatived by the following facts. In the first place, the sequence of the different steps, from the early prophase of the oögonium (Figs. 110–114) to the appearance of the chromosomes in the equatorial plate, is so close and so easily traced in detail that I have no doubt that they do actually succeed one another. For the evidence of this I refer the reader to the description and figures of the oögonial mitosis (pp. 336–341). Furthermore, the oögonia in mitosis very closely resemble somatic cells, whereas the oöcytes in the apparent prophase differ very markedly from them both in size and nuclear structure. In addition, the position of the different types of cells in the gonad is of some importance in this connection. As

previously stated, the oögonia lie in groups, chiefly basal, though occasionally in other locations; and all the mitotic figures found in a study of some two hundred sections of the female gonad lie in groups of these cells. The oöcytes showing the apparent prophases lie in entirely separate groups of five or ten cells each, usually crowded between the larger oöcytes, never (or very rarely) in connection with oögonia; in these groups not a single mitotic figure has been observed. The question as to the occurrence of the reconstruction phases is of importance, since this process is invariably a rather lengthy one, and these, though fairly common among the groups of oögonia, have never been seen among groups of the oöcytes. The weight of all this evidence is such that, although it is of course not absolute proof, it seems to me to establish beyond reasonable doubt the contention that the apparent prophase of the young oöcytes does not lead to any nuclear division whatever. As to the later fate of this phase of nuclear activity, it has been suggested that it may occur in cells, which, as the result of some disturbance, enter upon a prophase, and then degenerate, or at any rate proceed no further in development. There is no actual evidence whatever in favor of this view; and since, when nuclear degeneration does take place, the stages are always very abundant and conspicuous, it may be discarded without further discussion. I believe we must conclude that in this species, or at least in the specimens examined, this pseudoprophase of the oöcytes is a perfectly normal stage, which leads, not as might be expected to an ensuing nuclear division, but, through regressive changes, to a resting condition in which the nucleolus and chromatic bodies exhibit a condition very different from that seen in the resting stage which preceded it. I say "the specimens examined," for we must remember that in Thysanozoön, one of the two similar cases which I have been able to find in the literature of oögenesis, one observer, Selenka ('81), describes a very similar condition, while in other specimens of the same species, Schockaert (:01) was unable to find any trace of it.

We might naturally expect that it would be very difficult to distinguish the stages in regressive metamorphosis from the progressive stages of the apparent prophase. I believe, however, that the cell shown in Figure 129 represents a step in this process. In this specimen the dissociation of the segments into their component chromatin masses has progressed so far that their individual nature is almost entirely lost. At the same time the achromatic net has grown more complex, and the plasmosome has grown much larger. It is, however, sharply outlined, and shows no evidence that any of the chromatin bodies have coalesced

with it. The strongest argument against the contention that this cell represents a stage in the early prophase, is that both nucleus and cell bodies have grown very considerably while the nucleolus is of at least three times the bulk of the corresponding structure in cells such as are shown in Figures 120–123. Figure 130 (the nucleus only) shows the practical completion of the process. The chromatin structures are now entirely dissociated into a great number of granules, which lie at the nodes of an exceedingly delicate reticulum, and the nucleus is once more filled with dense and granular karyoplasm. The entire cell has increased still more in size, and several metaplasmic masses are to be seen lying in the cytoplasm in the neighborhood of the nuclear membrane. Of much interest from a general cytologic standpoint is the mode of reappearance of the karyoplasm. After all mitoses in Gonionemus this substance appears to be derived directly from the cytoplasm enclosed during the reformation of the nuclear membrane; in the present case, however, if my interpretation of the course of events be correct, we must suppose that it is the product of the transparent nuclear sap, being thus formed as the result of a series of changes directly the reverse of those which take place during the early prophase.

*Growth Period.* — At the close of the period of nuclear activity just discussed, — the "pseudoprophase," as I have called it, — the oöcyte enters upon the growth period so universal in the animal egg; its nucleus passes into the typical "resting" phase (Fig. 130), the chromatin structures being resolved into their component granules. A very important change occurs during the early part of the growth stage. Both reticulum and karyosomes almost entirely lose their affinity for nuclear dyes; and they retain this condition for a considerable period. The nucleolus, no doubt the direct descendant of the nucleolus of the pseudoprophase, is at first homogeneous and stains deeply, but as it grows larger one or more vacuoles appear in its substance (Fig. 130).

In the young oöcyte there is normally only one such nucleolus present, but at about the time that this becomes vacuolated there appear one or two other bodies, also of nucleolar nature, but of very different origin and composition (Fig. 131). These are always irregular in outline, somewhat spongy in structure, and stain very intensely with ordinary nuclear dyes. Usually only one is found, but occasionally two or even three are to be seen. They always lie at some distance from the chief nucleolus, though never in contact with the nuclear membrane. When first visible they are very small, hardly to be distinguished, except for their stronger staining reaction, from ordinary net-knots of the reticu-

lum, but with the growth of the nucleus they increase rapidly in bulk (compare Figs. 131, 132, with Figs. 133, 134). From their evidently compound nature (Fig. 133) it may be inferred that their growth is the result of an addition of granules or masses of similar composition to the original mass. Their more or less central position shows that they are intra-nuclear formations, and not, as Montgomery ('98[b]) suggests concerning nucleoli in general, of extra-nuclear origin; while all the evidence seems to indicate that they arise in very intimate connection with the nuclear reticulum. Following E. B. Wilson (:00), we may term them the "accessory nucleoli."

Shortly after the appearance of these "accessory nucleoli," the reticulum of the nucleus, which now measures about 14 $\mu$ in diameter, commences to regain its affinity for stains, and the chromatin commences to reassert itself. This shows itself in the appearance of a very large number of block-like, rather indefinitely outlined aggregations of deeply staining granules scattered throughout the nucleus (Fig. 131), which show an increasing affinity for the iron haematoxylin stain. There is no evidence that these receive any accession to their mass from either the chief or the accessory nucleolus, since neither of these two structures exhibits any change in structure or corresponding decrease in volume; we must therefore suppose that they are formed by a segregation of the chromatin granules formerly more generally diffused throughout the nuclear substance, and that their reappearance is due, not to the fact that they were previously masked by any other structures, but in part to their condensation and in part to changes in their chemical composition resulting in a stronger affinity for stains. These granular masses finally become very numerous, upwards of a hundred being often visible in a single nucleus; and they gradually arrange themselves in rows or strands (the commencement of this change is seen in the section represented in Figure 132), a process which continues until a variable number of independent strands result; these now stain strongly, especially in preparations previously treated with chlorine or hydrogen peroxide, and frequently exhibit Y- or V-shaped forms, as is seen in the nucleus of which a section is shown in Figure 133. The possibility that such figures may represent either a longitudinal splitting of the strands, or, on the other hand, their synapsis in pairs, will at once occur to the reader; but which, if either, of these explanations is correct can be settled only by tracing the formation of the individual chromosomes from the strands, and further discussion of this question is therefore idle until the late prophase of the first maturation division can be studied. The relation of the

strands to the surrounding and supporting karyoplasm is best shown on crushed specimens, where it is seen that, though rather firmly coherent, they are not sharply defined from the karyoplasm, but merge insensibly into it — a condition in contrast to that seen in the late prophases of the various mitoses in Gonionemus, in all of which the chromatin strands and segments are entirely distinct from the surrounding karyolymph. With the further growth of the nucleus the strands increase in length and also in number; the readiest explanation of this latter change is of course that the Y-shaped figures above described are due to longitudinal splitting. With this increase in number, and with the further growth of the nucleus as a whole, their relations to one another become so intricate that it is extremely difficult to determine whether they are still completely separate or form a loose and irregular net; a section of a nucleus showing this condition is represented in Figure 133, in which sections of various strands are seen, none of them probably being entire, and in addition the chief and the accessory nucleoli. With these changes the individual chromatin strands seem to diminish somewhat in thickness, though still preserving their granular consistency and showing that they are composed of series of separate block-like masses; the apparent dwindling is to be explained, I think, on the assumption that the rapid growth of the nucleus allows them to elongate more readily (Figs. 132 and 133). This is the most advanced stage to which I have been able to follow the changes in the chromatin structures, and since the germinative vesicle has now come to lie close against the periphery of the egg, I have little doubt that further stages must be sought after the eggs are set free.

Having thus briefly considered the genesis of the chromatin structures of the maturing germinative vesicle, and having seen that they are formed from the preëxisting nuclear reticulum without any apparent assistance from the nucleoli, I return to trace the further history of these latter bodies. The chief nucleolus at the stage last described (Fig. 131) consisted of a spherical mass of an apparently homogeneous granular ground substance without membrane, but enclosing one or more large vacuoles. With the further growth of the germinative vesicle, the nucleolus increases rapidly in bulk. Different specimens now exhibit a variable number of vacuoles; but since Pflücke ('95) has demonstrated in the living nucleus in gasteropods that one large vacuole may break up into several small ones, and then these may again unite into a single large one, variation in this respect cannot be considered of any great significance. Much more important, and at the same time of a more ob-

secure nature, are the differences seen in the ground substance of different examples. This is, as a rule, homogeneous; in many cases, however, (Figs. 132, 133), the vacuoles appear to be enclosed by walls of more deeply staining substance. Occasionally, also, the material enclosed by these denser walls is apparently of the same composition and density as the ordinary ground substance, instead of presenting the pale appearance of vacuoles (compare Fig. 137 with Fig. 138). In many cases the nucleoli enclose deeply staining strands and granules (Figs. 134, 135, 136), and in a few instances I have found specimens with no vacuoles at all, but traversed by a series of rather evident strands, variously thickened and connected with one another by deeper stained centres (Figs. 134, 135). These differences are not due to different degrees of development, for they occur in the smaller as well as in the more mature nuclei; and there is no evidence that such strands are stored chromatin, an interpretation placed upon similar appearances in echinoderms by Hartmann (:**02**) and Guenther (:**04**). It seems to me more probable that we have in Gonionemus such a condition as Montgomery ('**98**$^{b}$, p. 509) found in Polydora, where, "owing to the gradual confluence of the vacuoles, which thus produce anastomosing canals of vacuolar substance in the ground substance of the nucleolus, it is the true ground substance which represents a skein-like appearance."

In iron-haematoxylin preparations the chief nucleoli show great individual variation, being sometimes intensely stained, in other cases very pale, and occasionally almost transparent. But these differences are no more dependent on the degree of development of the nucleus as a whole, or on the condition of the chromatin structures, than are the variations in the nucleolar ground substance. To test this question further, I dismounted some of the preparations, washed out the haematoxylin, and restained in the Auerbach mixture, with the result that the same nucleoli which previously were stained hardly at all, were now stained as deeply as others of the same size; the only noticeable variation being that there seems to be a very slight loss of staining capacity corresponding to the increase in bulk. This fact is of great importance, for it shows that there is no loss of substance on the part of any of the nucleoli; and it emphasizes the danger of basing conclusions as to the relations of nucleoli to chromatin structures on iron-haematoxylin staining unchecked by other methods.

The accessory nucleoli, though varying considerably in size and outline, show no important differences in structure; all stain very deeply with ordinary nuclear dyes, being either of spongy or multiple nature,

and continuing to increase in bulk up to the latest stage examined (Fig. 137).

*Staining Reactions.* — The reactions to acid and basic stains of the various nuclear structures throw important light on their chemical relationships. For these experiments I have chiefly employed two fluids, the Auerbach mixture of acid fuchsin and basic methyl green, and a combination of acid Lichtgrün and basic safranin, the two mixtures being the exact counterparts of each other so far as concerns the chemical aspects of the two colors, red and green. They are thus well adapted to test conclusively whether or not the distinction between "erythrophilous" and "cyanophilous" substances is in any way fundamental in the present case. As might have been expected, it is not; the nuclear structures selecting exactly opposite colors, according as one or other of the solutions was employed. The Auerbach mixture gave rather more precise and consistent results, and I may therefore limit the description to the appearances obtained with it.

Treated by this method the reactions of the nuclei of the youngest oöcytes, of about the stage shown in Figure 120, are those typical of resting cells of Gonionemus, the karyoplasm, net-knots, reticulum, and membrane staining red or brownish, the nucleolus alone being green or blue. During the "pseudoprophase" of nuclear activity the chromatin segments take a very pure green; the karyolymph is unstained, and, to my surprise, the persistent portion of the nucleolus blue or green. During the regressive metamorphosis of the oöcyte the chromatin net gradually loses its affinity for the basic dye, and in the resulting, as in the preceding resting stage, all parts of the nucleus, except the nucleolus, stain red or brown, while the latter is green or blue. This condition, moreover, persists unchanged up to the latest stages studied, the chromatic strands, after their reappearance, staining very strongly with the red (acid) dye. The reader will recall that at this same stage the chromatin granules stain very strongly with haematoxylin, while the general nuclear ground substance remains almost unaffected by it, though it stains strongly with the ordinary plasma dyes, *e. g.*, eosin, Congo red, or fuchsin. This is a very interesting phenomenon, for it indicates that structures of undoubted chromatic nature within the nucleus may stain strongly, as these do, with haematoxylin, although they exhibit a chemical condition very different from that of chromatin in mitosis, as is shown by their reactions to acid and basic dyes; and it further emphasizes the necessity of employing both of these methods of investigation, the one for the study of the morphologic, the other for that of the chem-

ical constitution of the nuclear structures. The chief nucleolus continues to select the basic dye, and, so far as its finer structure is concerned, shows no differentiation, all its parts, ground substance, vacuoles, fibres, and granules, staining green. The accessory nucleoli, on the other hand, from the time of their earliest appearance to the most advanced stage examined, invariably stain strongly with the acid dye, and thus show a close relationship to the chromatin structures. These two classes of nucleoli are, then, very different chemically, as well as morphologically. All the evidence afforded by the history of the chromatin of the oöcyte nucleus goes to show that this substance makes no contribution to the growth of the chief nucleolus, yet the latter body exhibits throughout its entire history the reaction typical of chromatin in active mitosis. The accessory nucleoli, on the other hand, arise in close connection with, and probably as a result of, the metabolic activity of the nuclear reticulum, and their reaction is the same as that of the chromatin structures in the "resting" condition. The theoretic bearings of the occurrence of two distinct types of nucleoli can be discussed better in the general part of this paper, but I may here call attention to one conclusion of general and fundamental importance which is suggested by the staining reactions just described. This is, that in the growth stage, as in all the phases of progressive nuclear activity which I have studied in Gonionemus, mere segregation and condensation of the chromatin microsomes produces no change in their chemical reaction. The reversal of this reaction from the basic to the acid, which is characteristic of them when in active mitosis, is always simultaneous with a striking alteration in the general constitution of the nucleus. In every other prophase of mitosis this alteration consists of a dissolution of the karyoplasm, but whether we must seek the same determining factor in the later stages in the prophase of the first polar division can be answered only by further researches.

To examine further the nature of the nuclear structures, I made a series of experiments with chromatin solvents, and with a peptonic digestion fluid; since, however, I had at hand only fixed material, the results can hardly be expected to be conclusive. Treatment with fuming hydrochloric acid, even when prolonged until all chromosomes in mitosis are destroyed, leaves the chief nucleolus entirely unaltered, either in general structure or in staining capacity. The accessory nucleoli, on the other hand, as well as most portions of the chromatin strands of the oöcyte, are usually — not invariably — dissolved. This, of course, is further evidence of the close relations of the accessory nucleoli to the chromatin.

For experiments with digestion fluids I made use of only formalin material, since no alcoholic material was available, and albumins fixed with salts of the heavy metals, *e. g.*, osmium, are proverbially resistant to peptonic digestion. On such preparations treatment with the Kuskow mixture of pepsin and oxalic acid does not alter either class of nucleoli in the least, although cytoplasm is largely dissolved. So far, then, as tests for albumins in the nucleoli go, the results are negative ; but from the necessity of employing fixed material, they are not very important.

### D. Fertilization.

The material was in this case fixed with 40 per cent formaldehyde and stained with Grenacher's borax carmine, a combination which has proved very satisfactory owing to the transparency of the eggs. Without studying the living egg, which I have not done, it is impossible to follow the actual penetration of the spermatozoön; so, with the statement that in Gonionemus this always appears to take place just before the formation of the second polar spindle, I pass at once to the consideration of the nuclear phenomena involved. The earliest stage after penetration which I have been able to study is represented in Plate **7**, Figure 139. The egg is now surrounded by a thick, transparent, vitelline membrane (Fig. 139), to which supernumerary spermatozoa are often attached. This is fortunate, since it allows of a comparison between the sperm nucleus within the egg and the spermatozoa which have not succeeded in penetrating. The egg nucleus, now in the early metaphase of the second maturation division, lies midway between the centre and one pole of the egg, the spindle axis occupying a paratangential position. About equally distant from the opposite pole of the egg the sperm nucleus is clearly visible. It consists of two portions, a deeply staining, apparently homogeneous, triangular or conical portion, — the head, — which is still but slightly larger than that of the spermatozoön before penetration, and of an unstained, rather refractive middle piece, which, as has been shown, is of archoplasmic nature. No trace of the tail is to be detected. The sperm structure as a whole forms the centre of a series of astral radiations, the sperm aster, such as Maas ('**99**) has described in the fertilization of the sponge Sycandra. With the advance of the second maturation division, the sperm nucleus penetrates further and further into the egg, and at the same time commences to undergo the series of changes which are to fit it for actual conjugation with the egg nucleus. The middle piece soon disappears entirely ; but the radiations persist,

and now (Fig. 140), instead of centring about the sperm body as a whole, are directed toward a definite point at some distance from it. Although the aster is often very distinct, I have never been able to distinguish any definite body or granule at its centre which might be considered an undoubted centrosome. There is, however, in all cases a central, ill-defined, deeper staining region or sphere, in which the radiations are lost, and this may perhaps represent a centrosphere. By the time that the second maturation spindle has reached the anaphase, the sperm nucleus is as a rule deep in the substance of the egg, and in almost all cases the sperm aster which accompanies it has divided, the two daughter asters lying, not, as might be expected, in advance of it in its line of migration toward the egg nucleus, but on either side of it, as is shown in the particularly clear example represented in Figure 141. The time relations of the different steps in fertilization show great individual variation, and in some cases this division of the sperm aster takes place as early as the metaphase of the maturation division, while in others the sperm nucleus may be accompanied by only a single centre of radiation until it is almost in contact with the egg nucleus. Similarly, the changes in the sperm nucleus itself may take place earlier or later; but in any case, between the time of the division of the sperm aster and the re-formation of the egg nucleus, the sperm nucleus grows much larger, attaining to two or three times its previous bulk (compare Figs. 141 and 142). At the same time the sperm nucleus, which was previously homogeneous, now lies in a transparent, unstained area in the egg cytoplasm, loses much of its affinity for stains and grows distinctly granular.

After the metaphase the remainder of the second maturation mitosis is rapidly accomplished. The second polar cell is formed by the usual process of constriction, and the portion of the spindle figure remaining in the egg and its aster disintegrate and entirely disappear. The egg nucleus, now containing only half the somatic number of chromosomes (probably twelve) acquires a membrane. Then the chromosomes disintegrate into their component microsomes, leaving the nucleus in a reticular condition in which the chromatin is so finely diffused as to give it a granular appearance (Fig. 144). This stage is essentially like that following every adult mitosis in Gonionemus, except that no nucleolus is formed.

The egg nucleus now commences to move centrally toward the sperm nucleus, while the latter once more takes up its migration, with the result that the two nuclei come together in a somewhat eccentric posi-

tion in the egg. At the time of meeting the egg nucleus is almost invariably in the granular condition just described; the sperm nucleus, however, shows great variation in this respect. In the majority of cases it has perhaps three times the diameter of the mature sperm head before penetration, is of distinctly granular composition, has an oval outline, and is surrounded by a clear area (Figs. 143 and 144). At this stage the asters may or may not be visible, though, from conditions obtaining in earlier and later phases, we must suppose that they are normally present. When seen they lie as a rule on opposite sides of the nuclei, sometimes in or near their plane of contact (Fig. 143), but more often at nearly opposite sides of the egg nucleus (Fig. 144), showing now, as in later stages (Figs. 147 and 148), great individual variation in this respect. Since all trace of the achromatic figure of the second maturation spindle disappears previous to the re-formation of the egg nucleus, there is no doubt that these asters are the two sperm asters which have accompanied the sperm nucleus in its migration through the egg.

The sperm nucleus increases in bulk (Fig. 144), becomes more and more flattened against the membrane of the egg nucleus, and gradually changes its shape until it resembles a cap-like appendage to the latter (Fig. 145). It is still surrounded by a clear area, and is granular in appearance. The portion of the nuclear membranes separating the two nuclei now disappear, and the spongy substance of the sperm nucleus breaks up into a number of small chromatin bodies (Fig. 146), which collectively form a roughly spherical mass. Although accurate counting is impossible, there are certainly many more than twenty-four of these bodies. Meanwhile the chromatin of the egg nucleus, previously in a diffused condition, collects into an irregular reticulum composed of separate segments and thickenings connected by linin threads (Fig. 146). The two nuclei are now enclosed by a single continuous membrane. The fusion nucleus as a whole is now pear-shaped, the portion derived from the sperm forming the smaller end. The larger egg-nucleus portion frequently shows various constrictions, remotely suggesting the amoeboid form assumed by the egg nucleus in many other Metazoa, for example, in Cerebratulus (Coe, '99).

In the present example (Fig. 146) the two asters are clearly visible, one lying nearly in the plane of conjugation, the other near the opposite pole of the egg nucleus. The chromatin derived from the sperm nucleus now assumes more and more the appearance of an irregular network, until finally the paternal and maternal constituents are no longer sharply distinguishable from each other, although the fusion nucleus still pre-

serves in its form an indication of its double origin (Fig. 147). In this instance, then, there is no doubt that the membranes separating the two nuclei actually break down, allowing their contents to flow together and their networks to intermingle, so that this is not a case of mere apposition of nuclei.

Although this is the most common and therefore probably the typical form of nuclear union, in a few instances I have observed modifications of it which are due to the varying degree of differentiation attained by the germ nuclei at the time of their union. Although the portion of the membrane which at first separates them usually breaks down, it occasionally persists (Fig. 148). In this example the chromatin of the egg nucleus already has the usual form of a very loose threadwork, while that of the sperm nucleus, now nearly as large as the egg nucleus, forms a more compact network.

Although the chromatin of the egg nucleus at the time of contact is usually in the diffused condition, in one instance — a case of polyspermy (Fig. 142) — the chromatin net had reappeared by the time the two nuclei had come in contact. In another case this stage had been reached while the sperm nucleus still lay at some distance from the egg nucleus. The sperm nucleus, too, shows considerable variation in its nature at the time of meeting.

In the majority of cases the first cleavage nucleus shows no trace of its dual origin, the maternal and paternal constituents being indistinguishable. But from the occasional existence of such cases as those just described, in which the membrane separating the nuclei long persists (Figs. 148, 149), it seems possible that actual fusion may sometimes fail to take place. In any event, however, there is nothing in the history of the fusion of the germ nuclei in Gonionemus to preclude an equal distribution of both maternal and paternal chromosomes between the two daughter nuclei of the first cleavage.

Unfortunately, Gonionemus is not a satisfactory object on which to trace the history of the sperm aster and its relation to the asters of the first cleavage spindle, for it is impossible to detect the centrosome either in the middle piece of the sperm after penetration or in the sperm aster; while in the cleavage asters themselves the demonstration of any such structure is at best doubtful.

### E. The Cleavage Mitoses.

In the study of the nuclear cycle of any animal the history of the chromatin in the early cleavage mitoses is of great interest, both for the

light which it may throw on the rôle and subsequent fate of the maternal and paternal chromatin in the cleavage nucleus, and because of its importance in a comparison of the details of indirect division in larval cells and in adult tissues. The eggs of Gonionemus in the stages which I have at command have unfortunately proved to be very unsatisfactory for the solution of the first of these questions; but in the case of the second they have disclosed certain features with regard to the numerical condition of the chromosomes in the first, second, third, and fourth cleavages which seem to me so remarkable as to merit description, although some of the stages have not been worked out in as great detail as I could wish. On whole eggs as transparent as those of Gonionemus it is possible to trace fairly well the changes of the achromatic figure and of the chromatin in active mitosis; but the staining has proved unsatisfactory for a resolution of the finer details existing in resting nuclei, and in the prophase prior to the dissolution of the nuclear membrane. The sections were not more helpful in these stages.

### 1. *The First Cleavage.*

The first cleavage was studied chiefly on eggs fixed in 40 per cent formaldehyde and stained in borax carmine, only a very few corrosive-acetic preparations being available. The first cleavage nucleus is represented in Figure 150. It does not pass into a resting stage, nor is a nucleolus formed, but very soon after conjugation the nuclear membrane breaks down and the nuclear structures then lie free in the cytoplasm (Fig. 151). Previous to the dissolution of the membrane the chromatin was assembled in a loose and irregular threadwork (Fig. 150), but immediately after that event a process of condensation takes place, by which the threads thicken, become more clearly segmented, and considerably less numerous. This is a phase similar to that already described in both somatic cells and oögonia (pages 298 and 338) at a corresponding stage; it leads directly, through still further condensation, as in both those cell generations, to the formation of the definitive chromosomes (Fig. 152). These are rod-shaped, often somewhat dumb-bell-like, and usually lie more or less separated from one another, so that in two instances I have been able to count them with a fair degree of accuracy, the result in both cases being twenty-four. They now arrange themselves in an equatorial plate (Fig. 153). No centrosome can be distinguished, but there is to be seen at the centre of the radiations a deeply-staining homogeneous area (Fig. 153), which is perhaps comparable to a centrosphere.

For some reason polar views of this stage were exceedingly rare in the slides at my disposal, and owing to the elongate form of the chromosomes, it was impossible to count them with any accuracy in side views of the metaphase. But from the conditions obtaining in the late prophase, as just described, it is safe to conclude that they are present in the ordinary somatic number, probably twenty-four. Further evidence of this is seen during the anaphase. End views of this stage are comparatively common (Fig. 151), and it is possible on them to count the chromatic structures, though not with absolute accuracy. In all the cases examined the number was about twenty-four, though often apparently greater. The apparent excess is probably due to the elongated and constricted form of the chromosomes. I believe, therefore, it can safely be affirmed that the number of chromosomes appearing in the first cleavage spindle is the same as that characteristic of the somatic mitoses of adult tissues. This result is of course just what might naturally be expected, but it assumes special interest in view of the condition of affairs in the second cleavage spindle, to be described shortly. The later anaphase and the telophase present no features of peculiar interest. The nucleus passes into the resting condition, in which, however, no nucleolus is formed, and the achromatic figure entirely disappears. This figure (Fig. 153) differs very markedly from that seen in the somatic and in the male sexual cells, as will be at once perceived from comparison with the figures on Plates **1** and **2**, not only in the possession of prominent asters, but also in the condition of centrosome and spindle fibres as well, for in the present case the interzonal filaments, such a prominent feature of all mitoses in adult tissue, are even now hardly to be distinguished, and later, during the anaphase, break down and disappear. Moreover, the centrosome cannot be detected certainly. The asters themselves, however, persist until the commencement of the re-formation of the nuclei, after which they are no longer to be seen.

### 2. *The Second Cleavage.*

This division (Plate **8**, Figs. 155–158) was studied chiefly on whole eggs fixed in the corrosive-acetic mixture and stained in Grenacher's borax carmine, and on several series of sections; but no formalin material was available. The most cursory examination of the second cleavage spindle reveals a general aspect very different from that of the first cleavage, in the condition of both the chromatin structures and the achromatic figure. The second cleavage nucleus during its brief resting

stage is filled with dense karyoplasm, in which the chromatin persists in a finely diffused condition, no nucleolus being formed, and no archoplasmic structures whatever being at this time visible in the cell. After the nuclear membrane breaks down, the chromatic threads thicken and condense to form the individual chromosomes. Unfortunately, the nuclear contents at this stage are always so closely crowded that it has been impossible to count the chromatic elements; this has also been true of the equatorial plate stage, end views of which were for some reason exceedingly rare. In side views of this stage, however, it is evident that the chromosomes are very large and comparatively few in number (Fig. 155); in side views of the anaphase (Fig. 156) it is even more apparent that they are present in the daughter plates in a reduced number, apparently much fewer than the somatic twenty-four. With their migration toward the poles typical daughter plates are formed (Fig. 156), and in end views of these (Figs. 157 and 158), which are quite common, the chromosomes can be counted more easily and more accurately than in any other cells of Gonionemus. From a study of about ten such polar views two very important facts result; first, the number is without exception very much less than in the corresponding stage of somatic cells or of the first cleavage spindle; and secondly, the number of separate chromatin structures is variable, as are also their size and form. The actual counts made ranged from twelve, or possibly thirteen, to fifteen. In every instance the chromosomes are of different sizes, the larger ones being clearly of dual nature, as is shown in the two examples represented in Figures 157 and 158. In those cases, where the larger number, fourteen or fifteen, occur, two or more are invariably much smaller than any of the others (Fig. 158). This evidence indicates very strongly, I think, that the large chromatin bodies are double, each being formed by the union of two small chromosomes, and that this union is not very firm, the component portions of one or more of the dyads being readily separated. Briefly stated, then, the result of this division is that each daughter plate receives less than the somatic number of chromatic elements; a remarkable fact, the meaning of which can be discussed more intelligently after the description of the later cleavages.

### 3. *The Third and Subsequent Cleavages.*

The material proved insufficient for a study of the third cleavage, but when the fourth cleavage is reached, and, for that matter, all the subsequent mitoses, it is at once evident that the chromosomes are again

numerous, being present in the full somatic number. The nuclei, as well as the cells, are now of course much smaller than during the second cleavage, but just as in that case, the resting nucleus contains no nucleolus. I have not attempted to follow the finer details of the prophase, the available material being unsuitable for that purpose.

In the metaphase the chromosomes, which, as in adult somatic cells, are dumb-bell-shaped, arrange themselves with their long axes parallel to the future plane of division, so that in polar views their rod-like outlines are clearly evident, while in side views they present the appearance of minute spherical masses (Fig. 159), a condition similar to that seen in adult somatic cells, as well as in spermatogonia and oögonia. The splitting of the chromosomes is longitudinal, resulting in pairs of rod-like daughter chromosomes; since normally these are all visible in polar views of the late metaphase, it is then difficult, or impossible, to estimate the number of chromosomes actually taking part in the formation of the equatorial plate. Counts, however, of about fifteen such cases show numbers always in excess of twenty-four (27 to 38), so that it is altogether probable that they are present in the full somatic number. During the anaphase the chromosomes rotate so that their long axes coincide with the spindle axis. End views of such spindles (Fig. 160) are fairly common, and on them the counting of the chromosomes is possible with a fair degree of accuracy, the number being apparently twenty-four, exactly as in the adult somatic cells. It is also evident that the chromosomes are much smaller than at the corresponding stage of the second cleavage spindle (compare Fig. 160 with Fig. 157).

The later cleavage mitoses agree very closely in all their details with the fourth, and all exhibit the full somatic number of chromosomes. With the decrease in the size of the cells, however, the asters become less and less prominent, until finally, in larvae at about the stage of the first appearance of the primary tentacle, dividing cells no longer show any trace of them, and therefore resemble the ordinary somatic cells of adult tissues.

In view of Häcker's ('95) well-known discovery of the reduced number of chromosomes in the early segmentation stages of Cyclops, the fact that in Gonionemus, too, these structures are united, and even fused, in pairs in the early cleavage spindles, is of great interest. But the feature of the case, which, if normal, is the most remarkable, is the appearance of bivalent chromosomes of about half the somatic number in the *second* cleavage spindle, whereas in the *first*, where Häcker discovered the reduced number, the chromosomes are present in the full somatic number.

I say "if normal," because the two cleavages (the first and the second) were studied on material fixed by two very different reagents, and it is, therefore, possible that one or other of them represents a distortion of the natural condition. I feel confident that we cannot look upon the dyads in the second cleavage spindle as formed by a confluence of the chromosomes resulting from imperfect fixation, because in the later cleavage stages, fixed by exactly the same method, and even occurring on the same slides, no such confluence is ever seen, although the chromosomes are very much more crowded. It is, however, altogether possible that the use of strong formalin may result in the mechanical separation of such pairs of chromosomes, if they are not very strongly coherent; the more so, since its effect on the blastomeres themselves is to shrink and separate them, rendering their outlines in surface views particularly distinct. On this ground we might assume that in life the chromosomes in the first cleavage spindle, as well as in the second, are dyads and present in only one-half the somatic number; and that through the action of the fixing reagent the component individuals forming these dyads have been separated, so that in the preparations the chromosomes appear in the full somatic number. But, of course, to settle the question conclusively will require further research on material treated by other methods.

## V. General Discussion and Conclusions.

### 1. The Resting Nucleus.

The study of the resting nucleus of Gonionemus is of interest chiefly for its bearing on two topics: (1) the general changes taking place during the vegetative period, *i. e.*, between successive divisions; and (2) the relationship existing between the nucleolus and other nuclear structures.

The first of these I may pass over briefly, since it has recently been reviewed by Mathews ('98), Crampton ('99), Wilson (:00), and Rohde (:03). But for a clear understanding of the problems in hand a summary of their conclusions may be of value. The occurrence in the nucleus of two classes of bodies distinguished by diametrically opposite reactions toward stains was demonstrated by Heidenhain ('90) and Auerbach ('96). Shortly after, it was shown by the physiologic chemists Lilienfield ('93), Malfalti ('92), and others that this distinction has its basis in the fact that the affinity of chromatin for basic or acid dyes depends upon the degree to which its nucleinic acid is combined with albumin. It remained, however, for Mathews ('98) to correlate these discoveries with the different staining reactions exhibited by cell structures, and to reach a series

of conclusions which have formed the basis of all more recent studies along this line. Not only, says Mathews ('98), do basic dyes combine with chromatin, but they combine as readily, in acid solutions, with any other cell element whatever consisting of an organic acid in combination with a strong base: therefore, since there are many such combinations, *e. g.*, nuclein, hyaline cartilage, and mucin, similarity of staining is no proof of genetic relationship. And from this we must admit that "all conclusions in regard to the origin of cytoplasmic elements from chromatin, or their similarity to chromatin, based on staining reactions, are hence worth very little." (Mathews, '98, p. 452).

No doubt we must keep this warning constantly in mind in studying the cell as a whole; but in studying the nucleus alone, carefully chosen tests with acid and basic stains afford far the best index to the condition of nuclear elements which we can as yet command.

Although there occur two types of chromatin, as indicated by their sharply distinct staining reactions, yet, as Heidenhain ('90, '92) first showed, and as has since been thoroughly established, these two are morphologically indistinguishable. Nor is their chemical distinction permanent, for either one may become transformed into the other, such a change being exactly what takes place in Gonionemus during the regressive metamorphosis of the nucleus after division. E. B. Wilson (:00) has emphasized the relation between this change in staining reaction of chromatin and the periodic changes in its staining intensity, concluding from the very general occurrence of the latter that "the chromatin passes through a certain cycle in the life of the cell, the percentage of albumin, or albumin radicals, increasing during the vegetative activity of the nucleus; decreasing in its reproductive phase" (E. B. Wilson, :00, p. 340). More recently Rohde (:03) has brought abundant evidence to show that this conclusion is correct.

One feature of this cycle, briefly mentioned by Rohde (:03), still needs emphasis: this is, that a close correlation occurs between the degree to which the vegetative changes take place, and the degree of concentration of the chromatin. Rohde (:03) has shown that although an alteration in staining is very general, the degree to which this alteration progresses varies greatly in different nuclei. Thus, while in many nuclei, *e. g.*, those of oöcytes, vertebrate ganglion cells, and cells of the vertebrate pancreas, it progresses so far that reversal of staining reaction ensues, in others, such as ganglion cells of gasteropods, marrow cells of vertebrates, leucocytes, and gland cells in general, it is so limited that the chromatin structures continue to exhibit an affinity for basic stains.

Furthermore, we have seen in Gonionemus that, not only different nuclei, but different parts of the same nucleus may differ in this respect; for while the chromatin reticulum shows reversal, the chromatin "shell" of the nucleolus does not. In every case so far described the difference in behavior corresponds with a difference in morphologic conditions. Thus, reversal seems always associated with a diffuse, or finely divided condition of the chromatin, while maintenance of an affinity for basic stains is correlated with an occurrence of the chromatin in larger masses. Since reversal of staining depends upon an absorption of, and combination with, albumin (E. B. Wilson :**00**, p. 340), it is evident, as Rückert ('**94**) and E. B. Wilson (:**00**) have already noted, that it must be greatly facilitated by such an increase in the surface area of the chromatin as is afforded by a division of its substance into minute granules. It must, on the other hand, be correspondingly retarded by the grouping of the chromatin into larger masses. The effects of such difference in the morphologic condition of the chromatin are amply sufficient to account for the differences in its behavior towards stains.

### 2. The Nucleolus.

The literature dealing with the nucleolus in general has been reviewed in great detail by Montgomery ('**98**[b]), Lubosch (:**02**), Rohde (:**03**), and, in so far as concerns chromatin bodies described by this name, by Blackman (:**05**).

Very few students have devoted any attention to the coelenterate nucleolus. These few, though disagreeing as to various details, are agreed that the nucleolus is very intimately related to the chromatin structures. Thus Pfitzner ('**83**) describes the nucleolus in Hydra as contributing, in the prophase of mitosis, to the formation of the chromatin reticulum. Chatin ('**90**), on meagre evidence it is true, maintains that in sponges it consists wholly of chromatin. More important, because more detailed, are the observations of Guenther (:**04**). In Hydra, according to this author, the nucleolus of somatic cells is not homogeneous, as Pfitzner ('**83**) supposed, but is compound, consisting of a central pale staining mass surrounded by a shell of chromatin, exactly as I have described it in Gonionemus. The resemblance between the two forms is heightened by his description of a chromatin nucleolus in the spermatocytes. Furthermore, he is able to trace a complete series of stages from the chromosomes of the last spermatogonial mitosis to this chromatin nucleolus, showing clearly that a genetic relationship exists between the two.

Nucleoli of the compound type which occurs in these two coelenterates have been described in a few cases among the higher Metazoa. Thus, they are characteristic of leucocytes (Heidenhain, '92, '94), of epidermal cells of larval salamanders (Lavdovsky, '94), and of certain mammalian ganglion cells, gasteropod ganglion cells, and young ova of amphibians (Rohde, : 03), and especially of the spermatogonia of Paludina (Auerbach, '96). This last example shows a particularly close parallel to the condition in Gonionemus, since, according to Auerbach, the nucleolus, when treated with the Auerbach mixture of acid fuchsin and methyl green, shows a peripheral shell stained green and a central mass stained red. The similarity is still further emphasized by the fact that in Paludina, as in Gonionemus, the "shell" disintegrates during the prophase, and contributes to the formation of the chromatin reticulum. Such cases as these have led even Montgomery ('98[b], p. 507) to admit that "it would seem probable that the nucleolus in some cases has an envelope of chromatin forming a distinct capsule separated from the chromatin network of the nucleus." Yet he seems to have overlooked this as evidence of the relationship of nucleoli to other nuclear elements; for he states that he is unacquainted with any observations "which show that the nucleoli derive any part of their substance from the chromatin" (Montgomery, '98[b], p. 523).

Light has been thrown on this question by Rohde (: 03), whose methods, based on the use of basic-acid combinations of coal-tar stains, were much more precise than those of most of his predecessors. All nucleoli, says Rohde (: 03), originate within the nucleus. Furthermore, nuclei of all kinds originate by a concentration of nuclein — or chromatin — granules, and in their young stages exhibit an affinity for basic dyes. This is true of plasmosomes as well as of chromatin nucleoli: a fact which Rohde was able to establish by an extended series of observations. There is, then, as Flemming ('82) long ago surmised, no fundamental difference between the two classes of nucleoli, and instead of there being no genetic connection, as Montgomery ('98[b]) maintains, the relationship is of the closest, for plasmosomes are descended from chromatin nucleoli by a gradual alteration of their substance. Compound nucleoli, such as those mentioned above, which show a differentiation of their substance into an outer chromatin layer and an inner plasmatic mass, are nothing more than intermediate stages in the formation of plasmosomes. Such intermediate stages may, however, be permanent, as we have seen that they are in the case of Gonionemus. We have also seen that, in this form, the compound nucleoli are descended from nuclear elements

which show all the staining reactions typical of chromatin (p. 301), just as Rohde contends.

Accepting this view, as I think we must, we at once reach a satisfactory explanation for the fact that, while the somatic and spermatogonial nucleoli of Gonionemus are compound, the nucleoli of the spermatocytes are pure chromatin structures. Compound nucleoli occur in this animal only in cells — *e. g.*, somatic cells and spermatogonia — which divide at such long intervals that there is an opportunity for the formation, by secretion or otherwise, of plasmatic substance within the chromatin nucleolus. In the spermatocytes, however, the interval between divisions is too short to allow of this alteration, so that the purely chromatic condition of the nucleolus persists throughout the short vegetative period. Such chromatin nucleoli are, then, merely young stages of compound nucleoli and have no relation whatever to the structures in insect germ cells which have sometimes passed under this name (Paulmier, '99), but which are in reality specialized chromosomes. (See McClung, :02[a], :02[b], :05; Blackman, :05; and E. B. Wilson, :05, :06.)

Although my observations on Gonionemus strongly support Rohde's general conclusions, there is one point on which the evidence seems to me to oppose him. This is on the relationship between the chromatin and plasmatic substances of the nucleolus. Rohde believes that this relation closely resembles that between oxy- and basichromatin, and that the alteration by which plasmatic substance is formed from chromatin closely resembles that by which oxychromatin is formed from basichromatin. I believe, however, that in propounding this theory he has laid too much stress on the undoubted resemblance of the staining reactions, and has overlooked the much more essential evidence resting on the physiologic properties of plasmosome substance and oxychromatin. The latter substance, as we have seen (p. 360), is neither more nor less than chromatin in which the nucleinic acid is combined, for the time being, with an excess of albumin. But this condition is not necessarily permanent, as Heidenhain ('92, '94) demonstrated. Plasmosome substance, however, though a modification of chromatin, is a permanent one, for it is not capable of regaining its original characteristics. Furthermore, while oxychromatin takes part in the formation of the chromosomes, plasmosome substance appears never to do so, nor to have any functional activity in the nucleus. Instead, then, of being, like oxychromatin, a temporary state of chromatin, it is, I believe, a by-product, the nature of which is still unknown.

*The oöcyte nucleolus.*— The oöcyte nucleolus deserves separate consideration on account of its special features. This structure, according to the accounts hitherto published, shows a good deal of variation in different coelenterates. Thus, in Geryonia (Fol, '73), Eucope (O Hertwig, '78[b]), Spongilla (Fiedler, '88), Esperella (H. V. Wilson, '94), Aequorea (Häcker, '92[a]), Tubularia (Doflein, '96), Clava (Harm, : 02), and Gonothyrea (Wulfert, : 02), the nucleoli of the germinative vesicles are of a single sort, while in other genera, such as Aeginopsis, Nausithoe, Pelagia, Physophora (O. Hertwig, '78[b]), Hydra (Brauer, '91[a]), Rhodalia (Montgomery, '98[c]), Cordylophora (Morgenstein, : 01), and Gonionemus, there are two distinct classes of nucleoli which correspond closely in structure and staining reactions to the chief and accessory nucleoli so often described in the germinative vesicles of the higher Metazoa.

The general characters, and probably the relationships of these two classes of bodies seem, from the published accounts, very similar in the various forms in which they have been observed. But they have been variously interpreted. Thus Flemming ('82) doubts whether there is any valid distinction between the two; Brauer ('91[a]) and Floderus ('96) believe that accessory nucleoli are formed as buds from the chief nucleolus; but Häcker ('93) contends that there is no genetic connection between the two. List ('96) considers somatic and accessory nucleoli more closely related to each other than is either of them to the chief nucleolus. Montgomery believes that the two stand in no genetic relation to each other, in support of which view he points out ('98[b], p. 517) the probability that the structure interpreted as an accessory nucleolus in the older accounts was really nothing more than one of the vacuoles of the chief nucleolus, or extruded vacuolar substance of the latter.

Rohde's (: 03) observations, which are, in the main, supported by the conditions in Gonionemus, have thrown fresh light on this subject. There is, in Rohde's opinion, no more fundamental distinction between chief and accessory nucleoli than there is between the chromatin nucleoli and plasmosomes of somatic cells. On the contrary, the first two structures bear to each other much the same relationship as do the last two.

During the growth stage of the germinative vesicle both classes of nucleoli are "erythrophile," *i. e.*, combine with acid dyes. But the chief nucleoli originate, just as do ordinary plasmosomes, as nuclein structures, and, therefore, in their younger stages combine with basic dyes. This, however, is not the case with the accessory nucleoli. While the chief nucleoli are formed shortly after the last oögonial division, the latter do

not appear until much later, and from the beginning are usually "erythrophile."

In general, the character of the nucleoli in Gonionemus supports the foregoing generalizations, but the chief nucleolus in this animal shows one marked deviation from the usual condition, in that it retains throughout its history the affinity for basic dyes characteristic of its youngest stages. The chemical evidence, then, suggests that this structure retains its primitive composition of chromatin. But there could hardly be stronger morphological evidence that this is not the case than is afforded in its history. To begin with, we have seen that this nucleolus corresponds exactly with the plasmosome of somatic cells in origin, since it is merely the persistent central portion of a nucleolus of which the peripheral shell has taken part in the formation of the chromatin net of the pseudoprophase. Furthermore, it does not contribute in any way to the chromatin net of the germinative vesicle, but, on the contrary, appears to be wholly inert. Nor does there appear to be any good evidence that the deeply staining granules and fibres which occasionally occur in its ground substance are stored chromatin, as suggested by Carnoy ('85), Meunier ('86), Moll ('93), and Hartmann (:02); but it is more likely that these appearances in the vacuolated nucleolus are in reality caused by branching channels in the vacuolar substance, as Montgomery ('98[b]) believes to be the case in Polydora. If this view be correct, the staining reactions of the chief nucleolus of Gonionemus are most readily explained on the assumption that by-products of chromatin may sometimes be acid instead of basic.

Those authors who have traced the ultimate fate of the chief nucleolus in coelenterates have found that it either disintegrates (Brauer, 91[a], '91[b]; Maas, '99; Morgenstein, :01; Harm, :02; Wulfert, :02), or is cast out into the cytoplasm. All are agreed that it does not contribute in any way to the formation of the chromosomes. A diametrically opposite view, namely, that it does so contribute, is maintained by Hartmann (:02) and Guenther (:03[b]) for certain echinoderms. But their results have been criticized by Dublin (:05), who has shown from his own studies that observations based, as were theirs, on haematoxylin staining are of little value in this question. In Pedicellina he found stages where this method seemed to show that chromatin strands were being detached from the chief nucleolus, just as described by Guenther (:03[a]). However, Auerbach preparations proved that this was not the case, for, in Dublin's own words, "The nucleolus, at this point completely vacuolated, stains intensely red; and in most striking contrast, all the

chromosomes lying on its side are as intensely green." Pedicellina proved to be a very favorable object for study, because the chromatin retains its affinity for basic stains throughout the growth period of the oöcyte. And I think the evidence leaves no doubt that Dublin's conclusion, that it has no connection with the chief nucleolus, is correct. Indeed, there is, so far as I know, no conclusive evidence that the chief nucleolus in invertebrates ever normally contributes to the formation of the chromosomes of the first cleavage spindle.

### 3. The Chromatin Structures.

#### *a. The Spermatogonial Chromosomes.*

The formation of chromosomes by a linear union of smaller chromatin bodies has often been observed; but in very few cases do we know anything about the number of such chromomeres taking part in the formation of each chromosome. Boveri ('87) observed that in the first maturation division of the egg of Ascaris each element of the tetrad consists of six chromatin discs, while each chromosome of the germ nuclei is composed of at least twice as many. Brauer ('93) was able to trace these discs in more detail in the spermatogenesis of the same animal. In the spireme of the primary spermatocyte there are at first about forty such bodies, but before the segmentation of the spireme they are reduced to ten or twelve. After the segmentation each chromatin rod contains four or five, which finally fuse into a single mass. These observations have led E. B. Wilson (:00, p. 302) to the conclusion that the number of chromomeres is not constant for a given species.

There are, however, a few observations tending to show that the number may be constant. Thus Eisen (:00) describes the chromosomes in Batrachoseps as formed each by the union of six chromomeres, and Downing (:00, :05) finds that in Hydra each spermatogonial and each somatic chromosome is formed by the fusion of four chromomeres.

So far as I am aware, no case has ever been described in which on the one hand the spermatogonial chromosomes arise by a fusion of separate chromomeres, while on the other hand the somatic chromosomes are formed by a simple condensation of the chromatin segments, such as takes place in Gonionemus. It is difficult to reach a satisfactory explanation of this condition, but in this connection one cannot help recalling the occurrence elsewhere of plurivalent chromosomes, as in Ascaris, Artemia, and the germ cells of certain Hemiptera (McClung, :05).

It may be that the somatic chromosomes in Gonionemus are in reality

bivalent structures, the primitive univalent chromosomes being represented by the spermatogonial chromomeres. On this assumption we might interpret the constricted form of the somatic chromosomes as evidence of a bivalent nature; yet we must remember that such forms occur in many other animals, where there is no apparent evidence of double nature. Such an explanation does not seem unreasonable in view of the conditions in Artemia, in which, as Brauer ('94) has shown, the chromosomes, under certain conditions, regularly fuse in pairs.

*b. Numerical Reduction of the Chromosomes and Synapsis.*

The process of reduction in Coelenterata has been described by Boveri and Downing. Boveri ('90) found that in the hydromedusan Tiara there are fourteen tetrads in the first polar spindle; fourteen dyads in the second polar spindle, and fourteen univalent chromosomes in the egg nucleus. That this is a reduced number was shown by the fact that there are twenty-eight chromosomes in the first cleavage spindle. Downing (:00, :05) has described a very different condition in Hydra. According to his account there are forty-eight chromomeres in the spermatogonia. These chromomeres unite in fours to form twelve chromosomes. In the last spermatogonial division the daughter chromosomes are composed of only two instead of four chromomeres, so each daughter spermatocyte receives only twenty-four of these bodies, instead of forty-eight like its parent cell. In the first maturation division these chromomeres pair to form twelve chromosomes, which divide longitudinally. In the second maturation mitosis the division of the chromosomes is transverse, each spermatid receiving six chromosomes. According to this account Hydra exhibits an extraordinary condition, for while the number of chromosomes is reduced to one-half, the number of chromomeres is reduced to one-fourth. But neither Downing's description nor his figures seem altogether conclusive.

Guenther (:04) has traced the history of the chromatin in the spermatocytes of another species of Hydra with very different results. He finds that the chromatin instead of persisting as chromomeres, becomes diffused in the resting stage of the primary spermatocyte. The nuclear contents then contract into a mass containing all the chromatin of the cell; when this mass expands once more, the reduced number of chromosomes emerge from it.

Guenther's figures resemble so closely the contraction phases in the spermatocytes of Gonionemus that I have no doubt he was dealing with

a phenomenon of the same sort. But his account is not sufficiently detailed to indicate conclusively whether or not the contraction stages in Hydra are a normal part of the prophase. In view of these conflicting results I agree with Grègoire (:05, p. 296), that Hydra merits a fresh examination.

Similar contraction phases are common in the spermatogenesis and oögenesis of the higher Metazoa; thus they have been described in Ascaris (Brauer, '93, Sabaschnikoff, '97, Tretjakoff, :04), Lumbricus (Calkins, '95), copepods (Häcker, '92[a], Rückert, '94. Lerat, :05), ostracods (Woltereck, '98), Protracheata (Montgomery, :00), insects (Henking, '91, Montgomery, '98[a], Paulmier, '99, and others), molluses (Lee, '97), and in vertebrates (Moore, '95, Meves, '96, McGregor, '99, Eisen :00); likewise in the flowering plants (Sargant, '96).

Most investigators, following Moore ('95), have believed that numerical reduction of the chromosomes takes place during this condensation, but others — the most prominent being Lee ('97), McClung (:02[a]. :02[b]), and within the last year, Tellyschinczky (:05) — have maintained that these contraction phases are of purely pathologic nature, the condensation being in no way connected with synapsis. Tellyschinczky's results are especially important, since the material he used was the living cells from the testis of Salamandra, which he was able to study under the highest powers. Lee, moreover, was able to follow in Helix the actual formation of the "synapsis" artifact on living and slowly dying cells. Opinion, then, as to whether contraction stages are normal or artificial, is divided.

The fact that in Gonionemus and Helix they seem to be artificial is no conclusive argument that they need be so regarded in other forms. In all these cases, however, so far as I am aware, the same phenomenon of the "emptiness" of the nucleus accompanies the contraction phases. If we imagine the chromatin strands in Gonionemus as being under tension and ready to contract under the slightest stimulus, we need go but one step further to explain the situation in those forms, such as Ascaris and elasmobranchs, where the tension is so great that the contraction takes place without the necessity of an external stimulus. McClung's general contention, that such contraction phases are in all cases purely abnormal, seems to me unwarranted, although I agree with him, that too much stress has often been laid on the contraction as an essential feature of synapsis.

The most fundamental question in this connection is, whether in Gonionemus there is a true synapsis or pairing of the individual chromosomes;

and if so, when and how this pairing takes place. If, on the other hand there is no such synapsis, how are we to explain the appearance of the chromosomes in the equatorial plate of the first maturation division in only half the somatic number? It is certain, I think, that synapsis of adjacent pairs of chromosomes does not occur in the telophase of the last spermatogonial mitosis. Although Downing (: 05) describes such an event in Hydra, yet his figures, as well as the later history of the spermatocyte nucleus, which, in that form, then passes into a diffuse "resting" condition, show, I believe, that his interpretation of the appearances is erroneous. It is true that in Gonionemus the chromosomes as they approach the poles during the anaphase of the last spermatogonial division are at one period very closely crowded (Plate 3, Figs. 35, 36), but there is no evidence of pairing. This fact is of course in marked contrast to the important discovery of the very early occurrence of synapsis in chilopods (Blackman, : 05), Hemiptera (Sutton, : 02), Orthoptera (Baumgartner, : 04) and some Amphibia (Montgomery, : 03, McGregor, '99), and more nearly agrees with the earlier results of Rückert ('92) on selachians, Born ('94) and Fick ('93), and more recently Meves ('96), on Amphibia, and especially those of Calkins ('95) on Lumbricus. In all these cases where early synapsis has been conclusively demonstrated the chromosomes, although they may become more or less granular and irregular in outline, retain their individuality from one division until the next (Sutton, : 04, Baumgartner, : 04, Blackman, : 05). In Gonionemus, on the contrary, it is quite certain that during the resting stage of the spermatocytes the chromosomes, instead of persisting as such, disintegrate into their component granules, part of which mass together to form the nucleolus, and although this nucleolus is a chromatin structure, it is not a "karyosphere" in the sense in which Blackman (: 05) uses the term, for individual chromosomes do not enter into its make up, nor do they emerge from it during its disintegration. In the prophase of the first maturation division the chromatin forms a distinct chromatin net, in which, on account of the smooth and homogeneous character of the strands, we must suppose that the chromatin granules are very intimately united; much more so than is the case in either species of Hydra (Guenther, : 04, Downing, : 05). This net, or rather its chromatin component segments into structures which we may call chromomeres, which are only half as numerous as those of the spermatogonia, but each of which probably contains twice as much chromatic substance as does each of the latter. These chromomeres then unite two and two, and by their fusion form the definitive chromosomes, in half the somatic number, in this case

twelve. When we come to compare this process with the corresponding one in the spermatogonia, it is clear that the earlier prophase, and the occurrence of chromomeres from the fusion of which are formed half as many chromosomes, are practically identical in both cases. The formation of chromosomes by the pairing of distinct pre-existing bodies is in Gonionemus probably peculiar to these two cell generations; certainly it occurs neither in somatic cells nor in oögonia. Yet, since in the spermatogonia, in which the chromosomes appear in the full somatic number, the pairing can, of course, have nothing to do with synapsis or reduction, it seems too unreasonable to give it such significance in the spermatocyte.

Without entering here upon the theoretic side of the question, we may briefly consider two or three possible explanations of this reduction.

In the first place, if the primary spermatocytes were considered by themselves, apart from the preceding generation, it might naturally be supposed that, as in so many other metazoan cells, the chromatin structures resulting from the segmentation of the chromatin net were ordinary chromosomes in the usual somatic number (twenty-four), and that these then paired by a synaptic process to form the twelve bivalent chromosomes of the first maturation spindle. I repeat, did this cell generation stand by itself, or were it taken only in conjunction with the processes in somatic cells, such an explanation would seem altogether the most reasonable and natural one. But when we compare it with the corresponding stages of the spermatogonia, this solution does not seem to fit the case so well, for it gives to the process of pairing in the spermatocytes a significance which it cannot possibly bear in the earlier generation, although in both the actual course of events is, so far as can be seen, the same.

There is another explanation which seems to me to fit more fully the actual internal evidence afforded by the chromatin structures, although it is perhaps less easy to reconcile with the stages in the germ cells of other animals. This is, that a pairing of individual chromosomes does not occur at all in Gonionemus, but that synapsis occurs between the chromatic microsomes; and takes place while these are intimately associated in the homogeneous net. If we accept this solution, and to me, although the evidence is not altogether convincing, it seems the more probable one, it not only accounts for the occurrence of the chromosomes in the reduced number, but also offers a possible explanation for the formation in the spermatocytes of a continuous chromatin net, instead of the irregular and independent chromatin segments which occur in spermatogonia, oögonia, and somatic cells. Finally, there is still one more

possible explanation, though a rather artificial one; and that is, that the chromosomes both of somatic and of the earlier generations of germ cells are bivalent, and that it is to the "chromomeres" that we should turn for the univalent chromosomes. Evidence in favor of such a view is afforded by the apparently double nature and outline of the chromosomes, but I put it forward merely as a suggestion, which I am by no means prepared to support.

There can no longer be any doubt that in many animals — *e. g.*, worms (Calkins, '95), crustaceans (Nichols, : 02), Peripatus (Montgomery, : 00,) chilopods (Blackman, : 05), insects (Montgomery, '98[a]; Sutton, : 02; Baumgartner, : 02) — numerical reduction is the result of an end-to-end union of individual chromosomes. This view has been urged so forcibly, and it has fitted in with current theories of heredity so well, that the earlier view — namely, that reduction is caused by a segmentation of the spireme into half the somatic number of chromosomes — has been largely abandoned. Thus Blackman (: 05, p. 95) writes that "it [end-to-end union] may well be common to all sperm cells." But to apply this rule without reserve to all Metazoa is, in the present state of our knowledge, going too far. Although such a simple process of reduction as a mere breaking of the spireme into half the somatic number of segments may never occur, yet it is evident that the course of events in coelenterates and in Ascaris is very different, at least superficially, from that seen among the arthropods. After studying the careful and conscientious figures of Brauer ('93), Sabaschnikoff ('97), and Tretjakoff (: 04), it is hard to avoid the conclusion that in Ascaris, at least, the numerical reduction is the expression of a re-grouping of the chromatin granules, rather than a pairing of pre-existing chromosomes. Unfortunately, however, the process in Ascaris is obscured by the occurrence of a "contraction stage" at the critical period. In Gonionemus, likewise, the conditions are most readily explained on the assumption that there is a re-grouping of the chromatin granules. For it seems to me unreasonable to give to the pairing of chromatin structures in the spermatocyte a significance which cannot be attributed to the very similar process in the spermatogonia.

There is one very important point which appears from the evidence now at hand: in every case in which an early pairing of individual chromosomes has been demonstrated beyond doubt, — such cases are, with one possible exception, limited to the arthropods, — there is good reason to believe that the chromosomes preserve their morphologic individuality from the last spermatogonial until the first maturation division. This

has been demonstrated in Scolopendra by Blackman (:05), and in an even more convincing manner in insects by Sutton (:02) and Baumgartner (:04). In animals, on the other hand, in which the chromatin is entirely diffused during the resting stage of the primary spermatocyte, — *e. g.*, worms and coelenterates, — early synapsis has never been discovered, while in some of them, such as Ascaris and Gonionemus, it is doubtful whether any actual synapsis of individual chromosomes takes place at all. I realize, however, that the evidence on this point is by no means conclusive; it is only by a renewed examination of molluscs, worms, echinoderms, and coelenterates that a sound decision can be reached.

### *c. The Pseudoprophase of the Oöcyte.*

The occurrence of an abortive attempt at mitosis in the development of the female germ cell, although unusual, is not unparalleled. Selenka ('81) described a similar process in Thysanozoön, in which the nucleus of the young oöcyte undergoes the ordinary series of prophase changes, proceeding as far as the formation of the achromatic spindle. But after the metaphase regression follows, with the result that the nucleus returns to the "resting" condition without dividing. Selenka has shown beyond doubt that he was not examining any part of the first maturation division, for he remarks that while the abortive prophase takes place in the ovary, the maturation division does not occur until after the egg is laid. More recent students of the same animal — van der Stricht ('96), Schockaert (:01) — have found no trace of any such abortive prophase, so that it is now doubtful whether it is normal in this species.

A second instance of similar nuclear activity has been described by Woltereck ('98) in the parthenogenetic eggs of Cypris. In the youngest oöcyte the chromatin is diffuse, but after a short "resting" period it collects into strands, which contract into a dense mass at one side of the nucleus. Later these strands emerge and segment into the somatic number of chromosomes. Instead of proceeding further in mitosis, the nuclei then undergo regression, which eventually results in their returning to the finely reticular "resting" condition. So far as I know, these two are the only accounts of any such process, for although Woltereck ('98) sees a similarity in the conditions observed by Häcker ('92b) in Canthocamptus, there is in this case a persistence of chromatin rods from the last oögonial division throughout the growth period of the oöcyte, a state of affairs very different from an abortive mitosis. As to the significance of this stage, we can conclude with Woltereck ('98) that

it represents a mitosis which, for some reason or other, is never completed. Further than this we can say little, except that, as Woltereck has shown, it can have nothing to do with reduction, because in the parthenogenetic eggs of Cypris the chromosomes appear in the full somatic number in the first polar spindle.

### *d. The Chromosomes of the Cleavage Spindles.*

One of the most interesting features which have appeared from this study is the occurrence in Gonionemus of a reduced number of chromosomes in the early cleavage spindles. I have already (p. 360) called attention to the possibility that this condition may perhaps not be typical of this animal, but may be in some way abnormal. To settle this, however, requires renewed examination of fresh material of this same form. Till such material is available I prefer to regard the process as the normal one, the more so since there is nothing in the preparations to suggest that it is in any way abnormal.

A similar case has been described in Cyclops by Häcker ('95), who discovered that the early cleavage spindles show only half the somatic number of chromosomes. Each of these chromosomes, however, is shown by its form to consist of two ordinary chromosomes joined end to end, and is therefore bivalent. The reduced number of chromosomes is permanently retained by the primordial germ cells, which are differentiated as early as the eight-cell stage. But the ordinary somatic cells after this stage acquire the full somatic number. This alteration, as E. B. Wilson (: 00, p. 274) remarks, "must consist in the dividing of each bivalent rod into its two elements." Although no exact parallel to this condition seems ever to have been described, vom Rath ('93, p. 106, footnote) states that he has found nuclei with only half the somatic number of chromosomes in blood cells and cells of the primitive kidney and midgut of larval salamanders.

Although the conditions in Gonionemus closely resemble those in Cyclops, an apparent difference is found in the fact that in Cyclops there are only half the somatic number of chromosomes in the first cleavage, while in Gonionemus there are the full number in this division. But this divergence is more apparent than real, since it is caused merely by a variation in the time when the pairing of the chromosomes takes place. Häcker's ('95) figures show very clearly the exact method of this pairing in Cyclops. In his Figure 45 — a polar view of the early metaphase of the first cleavage immediately after the disappearance of the

membranes of the two pronuclei — one of the two chromosome groups consists of twelve separate bodies, while in the other there are only six, which from their shape are evidently bivalent. At a slightly later stage shown in his Figure 44, there are in all twelve bivalent chromosomes. From these same figures there appears one very significant fact; the pairing takes place between members of the same chromosome group, so that both members of each bivalent chromosome are either paternal or maternal in origin.

In attempting to explain this temporary reduction in the number of chromosomes in the early cleavage spindles, we are on very uncertain ground. E. B. Wilson (:**00**, p. 274) maintains for Cyclops that "we have here a wholly new light on the historical origin of reduction; for the pseudo-reduction of the germ nuclei seems to be in this case a persistence of the embryonic condition." But this conclusion will not apply at all to Gonionemus. In this animal all the embryonic nuclei acquire the full somatic number of chromosomes after the fourth cleavage. Moreover the germ cells are not differentiated until after a long series of cell divisions. And they also, when first formed, exhibit the full somatic number of chromosomes. Therefore in this animal the pseudo-reduction in the cleavage nuclei has no connection whatever with the pseudo-reduction in the germ nuclei.

### 4. The Metamorphosis of the Spermatid.

The recent discussions of this subject by Meves (:**02**), Korschelt und Heider (:**02**), and Waldeyer (:**01**) have been so thorough as to make any detailed consideration of it unnecessary here. I shall therefore limit myself to a brief comparison between the process in Gonionemus and that which has been described for other coelenterates.

*a. The centrosome.*— The only author who has traced the fate of this structure in the coelenterate spermatid is Görich (:**03**[a], :**04**). In Aurelia and Sycandra, according to Görich, there are two centrosomes in the spermatid. These at first lie side by side on the cell margin, but one soon migrates inward to the nucleus, remaining connected, however, with the outer one by an axial strand or filament. According to Downing (:**05**), metamorphosis of the spermatid in Hydra follows a different course, for he found only one centrosome, which lay at the cell margin. This result agrees with the earlier studies of Aders (:**03**) on Aurelia. But Görich (:**04**) has shown that Aders's observations were very incom-

plete. And since Downing's figures suggest that he saw only a few of the stages in the metamorphosis, it is not improbable that a division of the centrosome, and migration of one of the resultant halves takes place in Hydra, as it certainly does in Gonionemus. Görich (:03[a]) states that the history of the centrosome is the same in various other hydroids and medusae; and since I have myself been able to confirm his account in Aurelia and Metridium, as well as in Gonionemus, it seems safe to conclude that the process observed in the latter animal is the one general among coelenterates.

*b. The archoplasmic structures.*— Our first accurate knowledge of these structures in this group is due to Pictet ('91), who discovered a homogeneous mass lying beside the nucleus in the spermatid of the siphonophore Halistemma. This mass he believed to be formed by the coalescence of granules which "sont . . . des produits d'élimination de la dernière division caryocinétique des spermatocytes." In Gleba, likewise, there is the same "Nebenkern," and also in the spermatozoön a spherical middle piece shown by its staining reactions to be of similar nature. Ballowitz ('94) has described much the same condition in the actinian Tealia, finding a "Nebenkern" and also one or two smaller masses lying near the point of origin of the tail. These observations were extended by Retzius (:04, :05), who found that the middle piece in several other coelenterates — *e. g.*, Cyanea, Tubularia, Clava, Sertularia — consists of two or more spherical archoplasmic masses. With this account the condition in Gonionemus closely agrees. Görich (:03[a], :04) seems to have entirely overlooked this substance in the spermatid of Aurelia and Sycandra. But since I have myself demonstrated, by use of the iodine method, that the archoplasm plays the same rôle in Aurelia that it does in Gonionemus, I attribute his failure to detect it to his dependence on haematoxylin staining and on such plasma dyes as Bordeaux red. Taking these various observations together, we can safely conclude that the middle piece among coelenterates in general is an archoplasmic structure.

Most authors who have traced the origin of the middle piece have maintained that it is descended from the interzonal portion of the spindle. This view is maintained especially by Wilcox ('95, '96); Erlanger ('97). Paulmier ('99); and Baumgartner (:02). Gonionemus, however, shows a divergence from this type, in that the archoplasmic middle piece is derived, at least in part, from the remnants of the *polar portion* of the spindle, thus closely paralleling the conditions observed by Calkins ('95) in Lumbricus.

The origin of the acrosome has never been traced in any coelenterate, although this structure has been observed in various members of the group by Pictet ('91); Ballowitz ('94); Aders (:03); Retzius (:04, :05); Görich (:03[a], :04); and others. Its staining reactions, however, show clearly that it also is of archoplasmic nature, as is now so generally considered the case among the higher Metazoa. (See Meves, '97, '98, '99, :00; Wilcox, '96; McGregor, '99; Paulmier, '99; Tönniges, :02; Blackman, :05.)

*c. The axial filament.*— In Gonionemus the evidence is very strong that the axial filament of the tail is an outgrowth of the substance of the centrosome: a result indicated, though less conclusively, by the observations of Görich (:03[a], :04) on Sycandra and Aurelia. Of especial interest in this connection is the occurrence in Gonionemus of giant and multiple spermatids of a type closely resembling those described by Paulmier ('99) in Anasa. In the latter case, according to Paulmier, a separate axial filament arises in connection with each centrosome, although there is but a single mass of archoplasm. This has seemed to E. B. Wilson (:00) conclusive evidence that the filaments are actual outgrowths of the centrosome. And this is entirely corroborated by Broman (:02), who has found that in abnormal spermatids of mammals and elasmobranchs one filament is developed in connection with every centrosome, no matter how many nuclei or spheres there may be in the cell. Still further support is lent to this view by the discovery of abortive filaments in connection with the centrosomes in the spermatocytes of elasmobranchs (Moore, '95), Lepidoptera (Meves, '97, :00; Henneguy, '98); and Gonionemus (pp. 322); while Meves (:00) has shown that in Pygaera these filaments — of which there are two in the secondary spermatocyte, one attached to each arm of the V-shaped centrosome — may actually persist to form the tail filaments of the spermatozoa.

It is probable, then, that a genetic relationship between centrosomes and axial filaments is very general, as has already been pointed out by Meves (:02), and Korschelt und Heider (:02). But such a relationship is not universal, for Tönniges (:02), P. et M. Bouin (:03) and Blackman (:05) have shown that the filament in myriapods is formed from the archoplasm, independent of the centrosome; a conclusion which is certainly true for the "pseudoaxial filaments" described by Blackman (:05), for these cannot possibly have any connection with the centrosome.

### 5. Fertilization.

Both methods of union of the germ nuclei — fusion and mere apposition — have already been observed among coelenterates. The first has been described in greatest detail by Boveri ('90). In Tiara, according to this author, the sperm nucleus is a small, compact chromatic mass, surrounded by a clear area; a condition much like the one seen in Gonionemus at a corresponding period (p. 354). This clear area then becomes continuous with the "vacuolar space" of the egg nucleus, and both maternal and paternal chromatin become enclosed by a single membrane. Boveri furthermore made the extremely important discovery that the maternal chromosomes are formed before the paternal chromatin mass undergoes any changes, thereby corroborating for coelenterates the earlier results of Mark ('81), and especially van Beneden ('83), that the chromosomes of the first cleavage spindle are purely maternal or paternal.

A similar inclusion of the paternal chromatin mass by the egg nucleus occurs in Gonothyrea (Wulfert, :02) and, as I have shown, it is the usual mode of union of the germ nuclei in Gonionemus. In other coelenterates in which fusion takes place — *e. g.*, Hydra (Brauer, '91[a]); Tubularia (Brauer, '91[b]); Clava (Harm; :02), and Cordylophora (Morgenstein, :01) — the two germ nuclei are of about equal size at the time of their union. Fusion is not, however, invariable, for mere apposition of the germ nuclei occurs in Aequorea (Häcker, '92[a]), as well as, exceptionally, in Gonionemus.

The occurrence of two alternative methods of nuclear union in one species, such as is seen in Gonionemus, although uncommon, is not unparalleled. So long ago as 1887 Boveri ('87) described a similar condition in Ascaris, finding that fusion occasionally replaces the more usual apposition; and more recently Wulfert (:02) has described a similar state of affairs in Cordylophora. E. B. Wilson (:00) has shown that there is no essential difference between the two methods of nuclear union; the occurrence of one or the other depending on the time which elapses between the penetration of the spermatozoön and the meeting of the nuclei. "On general grounds we may confidently maintain that the distinction between the two . . . is due to corresponding differences in the rate of development of the germ nuclei, or in the time that elapses before their union" (E. B. Wilson, :00, p. 205). It is, I believe, the first of these two factors that determines the mode of union in Gonionemus, fusion taking place when the sperm nucleus is small at the time of union, apposition when it is of nearly the size of the egg nucleus.

## VI. Summary.

*Somatic mitosis* (Figs. 3–14). — The "resting" somatic nucleus of Gonionemus is filled with dense karyoplasm; there is an achromatic reticulum with nodal thickenings or karyosomes, and a single large nucleolus, consisting of a peripheral shell of chromatin and a central mass of plasmatic substance. Many of the achromatic threads radiate from the nucleolus. At this stage all portions of the nucleus except the nucleolar shell combine with acid dyes, the latter combining with basic ones.

In the prophase the karyosomes increase in size, and reverse their staining reaction simultaneously with the disappearance of the karyoplasm, while the nucleolar shell breaks down and contributes its substance to the chromatin structures. The karyosomes, together with the masses of chromatin derived from the nucleolar shell, become concentrated along the courses of the achromatic strands, forming separate chromatin segments. These segments contract and form the dumb-bell-shaped chromosomes without being metamorphosed into a continuous spireme thread.

The somatic number of chromosomes is probably twenty-four.

No centrosome or archoplasmic structures are visible in the cell until the metaphase, when the spindle figure is formed. This is extremely simple, there being no trace of astral radiations.

The centrosome is a minute granule at the focus of the spindle fibres. After the anaphase it can no longer be detected.

In the anaphase interzonal filaments are formed, which are stouter and more clearly granular than the spindle fibres.

In the reconstruction of the nucleus the nucleolus is formed by a condensation of chromatin granules. This body is at first entirely homogeneous, and only when it has attained its full size does the plasmatic substance become differentiated in its central region.

*Spermatogonia* (Figs. 15–38). — In the spermatogenesis of Gonionemus the growth period takes place in the spermatogonia.

During the "resting" stage the nuclei are similar in structure and staining reactions to those of somatic cells; the chromatin is diffused and the nucleolus is of the compound type. The cytoplasm often contains metaplasmic masses, but no archoplasm can be detected, and it is doubtful whether a centrosome is present.

In the early prophase the karyosomes, as in somatic cells, reverse their staining reaction simultaneously with the disappearance of the

karyoplasm. The nucleolar shell contributes to the chromatin structures, but the central plasmosome persists until the breaking down of the nuclear membrane. The chromatin becomes condensed into forty-eight spherical chromomeres, which are connected by linin strands.

After the breaking down of the nuclear membrane the twenty-four chromosomes are formed by the union in pairs of the chromomeres. Their constricted form results from this double origin. In division each chromomere is divided lengthwise.

The achromatic figure, which is formed during the metaphase, has no astral radiations. Though the centrosome, which is a minute granule, disappears in the late anaphase, the stout interzonal filaments often hold the daughter cells for a long time in connection.

The chromosomes do not pair in the telophase, but become connected into an irregular network, and eventually break up into their component granules.

The nucleolus is formed by a condensation of the chromatin granules.

*Primary spermatocytes* (Figs. 39–70). — In the "resting" stage the chromatin is diffused. The nucleolus is not compound, but is a purely chromatin structure, which breaks up in the prophase and contributes to the formation of the chromatin net. The staining reactions of the nucleus are those typical of somatic cells and spermatogonia, except that the entire nucleolus combines with basic dyes. During the prophase contraction of the chromatin structures often occurs, but this is believed to be artificial and not to represent a normal stage in spermatogenesis.

In the normal prophase the chromatin becomes condensed into a smooth, homogeneous net of few strands, which, instead of being metamorphosed into a continuous spireme thread, probably segments into twenty-four chromatin masses. Upon the disappearance of the nuclear membrane these masses pair, forming twelve chromosomes.

Apparently there is no formation of tetrads.

The spindles of the first maturation mitosis are of two types. In the more usual one the chromosomes become dumb-bell-shaped and divide by a gradual drawing apart of their thickened ends. In the rarer type division is rapid and irregular, and "dumb-bells" are not formed; but in both the daughter chromosomes are much larger than those of the spermatogonial division.

It is impossible to say certainly whether the first maturation mitosis is an equation or a reduction division.

*Secondary spermatocytes* (Figs. 71–87). — The secondary spermato-

cyte is much smaller than the primary spermatocyte; its nucleus during the "resting" stage closely resembles that of the primary spermatocytes; the achromatic reticulum bears nodal thickenings, and the nucleolus is a purely chromatin structure. Its staining reactions are those typical of other cell generations, and the usual reversal of reaction takes place on the part of the karyosomes in the early prophase.

Contraction phases are common in the prophase of this as well as of the preceding mitosis, but are believed to be purely artificial.

The chromatin becomes condensed into an irregular network; before the breaking down of the nuclear membrane this segments into twelve chromosomes without any intermediate formation of chromomeres.

The spindles are very small; no "dumb-bells" are formed, since the splitting of the chromosomes is rapid and simultaneous, and the daughter chromosomes are very much smaller than those of the preceding division.

It is impossible to say certainly whether the division is reducing or equational.

The centrosome does not disappear in the anaphase, as is the case in all other mitoses in Gonionemus, but persists to form the spermatid centrosome.

*Metamorphosis of the spermatids* (Figs. 88–108). — The spermatid centrosome, which lies at the cell margin, divides, and one-half migrates inward to the nucleus, finally being flattened against the nuclear membrane. It remains connected with the distal centrosome by an axial filament. The tail filament is believed to be an outgrowth of the distal centrosome.

The interzonal bridge connecting pairs of daughter spermatids breaks down. The centrosome migrates along the margin of the cell until it comes to lie in the prolongation of the long axis of the nucleus. The remnants of the interzonal filaments remain attached to the nucleus, but the remnants of the polar portion of the spindle always lie in the neighborhood of the centrosome.

The interzonal remnants disappear, and afterwards two archoplasmic masses are to be seen in place of the polar remnants, one lying on either side of the axial filament. These increase in size, and form the sheath of the middle piece.

The acrosome is a sphere of archoplasm, which arises after the disappearance of the interzonal remnants; its origin is doubtful.

In addition to the normal spermatid there are spermatids showing abnormalities of two kinds caused by incomplete spermatocyte divisions.

These are (1) "giant" spermatids, resulting from mitosis in which neither nucleus nor cell body has divided; (2) "multiple" spermatids, resulting from divisions in which the nuclei alone have separated.

These plurivalent spermatids have from one to four centrosomes according as they correspond to from one to four normal spermatids. There is a tail filament in connection with each centrosome irrespective of the numerical conditions of nuclei or of archoplasmic masses.

*Oögenesis.*— Oögonia (Figs. 109–119). — The division of the oögonia is of the ordinary somatic type, the chromosomes being formed by the direct condensation of the chromatin segments without any intermediate formation of chromomeres.

*Oöcytes* (Figs. 120–138). — The young oöcytes undergo a series of prophase changes which do not lead to nuclear division. The nucleolar shell breaks down and contributes to the chromatin reticulum. The chromatin condenses into separate "beaded" segments formed by the union of varying numbers of karyosomes. The central portion of the nucleolus persists, but, unlike the corresponding plasmosome of the spermatogonia, it combines with basic instead of acid dyes.

The chromatin segments then become irregular and break down, and the nucleus, instead of undergoing division, returns to the "resting" condition, in which the chromatin is diffused. The central portion of the nucleolus persists, and develops into the chief nucleolus of the growth period of the oöcyte.

*Growth period* (Figs. 129–138). — In the early growth period the chromatin largely loses its staining capacity. Later the chromatin reasserts itself and appears in block-like masses of granules. These blocks arrange themselves in strands, which, though they stain deeply with haematoxylin, select acid dyes; they frequently form Y- or V-shaped figures.

The chief nucleolus grows, becomes vacuolated, and frequently shows strands and granules in its ground substance. It takes no part in the formation of the chromatin strands. Throughout its existence it selects basic dyes.

During the early growth period accessory nucleoli are formed. These arise in connection with the nuclear reticulum; they are irregular in outline, and agree in staining reactions with the chromatin strands. During the later growth period they increase in size. These nucleoli select acid dyes.

*Fertilization* (Figs. 139–149). — The sperm structure after penetration consists of head and middle piece; it is surrounded by astral radia-

tions. Later the middle piece disappears and the radiations centre at a point at some little distance from the sperm nucleus, but no sperm centrosome could be detected. The aster divides and forms two asters, which accompany the sperm nucleus in its migration through the egg.

The egg aster disappears at the close of the second maturation division.

Nuclear union takes place either by fusion or by apposition; the determining factor is believed to be the relative sizes of the nuclei at the time of their union.

*Cleavage* (Figs. 150–160). — In the first cleavage spindle — as seen in formalin preparations — there are the full somatic number of chromosomes.

In the second cleavage there are a reduced number of chromosomes, each of which is a bivalent structure resulting from the pairing of univalent chromosomes.

The number of chromosomes in the third cleavage has not been observed, but in the fourth and subsequent cleavages all nuclei have the full somatic number of chromosomes.

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## EXPLANATION OF PLATES.

All drawings were made with the aid of a camera lucida. All the figures are reproduced without reduction. In the description of each figure the magnification is given.

The magnification of 2600 diameters was obtained with a Bausch and Lomb $\frac{1}{12}$ inch homog. immersion objective and $\frac{1}{2}$ inch eyepiece at a projection distance of 450 mm.; that of 1780, with $\frac{1}{12}$ objective and 1 inch eyepiece, projection distance 450 mm.; that of 900, with $\frac{1}{12}$ objective and 2 inch eyepiece, projection distance 350 mm.; and that of 500, with a D objective of Zeiss, 1 inch B. & L. eyepiece, projection distance 350 mm.

### ABBREVIATIONS.

| | |
|---|---|
| *ec'drm.* | Ectodermic covering cells of gonad. |
| *en'drm.* | Endoderm of radial canal. |
| *ms'gl.* | Mesogloea. |
| *o'cy.*$^1$ | Pseudoprophase of oöcyte. |
| *o'cy.*$^2$ | Growth stage of oöcyte. |
| *o'go.* | Oögonium. |
| *sp'cy.*$^1$ | Primary spermatocyte. |
| *sp'cy.*$^2$ | Secondary spermatocyte. |
| *sp'd.* | Spermatid. |
| *sp'go.* | Spermatogonium. |
| *sp'zo.* | Spermatozoa. |

## PLATE 1.

All figures are from sections.

Fig. 1. Section of female gonad crosswise to radial canal, showing the general arrangement of the developing germ cells. × 500.

Fig. 2. Section of male gonad crosswise to radial canal, to show general arrangement of the various cell generations. × 500.

Fig. 3. "Resting" stage of endodermal cell. A part only of the cytosome is shown. The nucleus is in the reticulate condition. × 2600.

Fig. 4. Early prophase of endodermal cell. The karyosomes have become prominent, and the karyoplasm less dense. × 2600.

Fig. 5. Later prophase. The karyosomes are larger and the nucleolar shell has commenced to break down. × 2600.

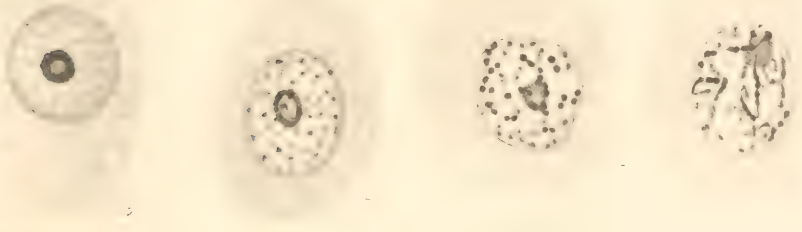
Fig. 6. Still later prophase of endodermal cell. The karyosomes have become condensed into definite segments. × 2600.

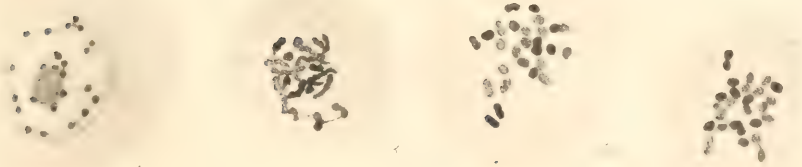
Fig. 7. Part of a crushed nucleus in prophase, showing the breaking down of the nucleolar shell. The plasmosome is vacuolate; only a few of the karyosomes are shown. × 2600.

Fig. 8. Late prophase. The nuclear membrane has disappeared. Several chromosomes have been formed. × 2600.

Figs. 9, 10. Metaphase, seen in polar view. There are about 24 chromosomes in each nucleus, many of them dumb-bell-shaped. × 2600.

Fig. 11. Side view of metaphase of endodermal cell. The centrosomes are now visible. Several chromosomes have commenced individually their migration toward the poles. × 2600.





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## PLATE 2.

All figures are magnified 2600 diameters.

Figs. 12–14. From sections.

Figs. 15–31. From isolated cells.

Fig. 12. Anaphase of endodermal cell. Stout, granular interzonal filaments connect the two chromosome groups. The centrosomes have disappeared. The chromosomes are closely compacted.

Fig. 13. Telophase. The chromosomes have become connected into an irregular network, and the nuclear membrane has re-formed.

Fig. 14. The earliest stage of the reconstruction when the nucleolus can be detected.

Fig. 15. "Resting" stage of spermatogonium. The nucleolus is compound. The cytoplasm contains metaplasmic masses.

Figs. 16, 18. Spermatogonia. The cytoplasm contains granules, which may be centrosomes.

Fig. 17. Spermatogonium with two nucleoli.

Fig. 19. Early prophase of spermatogonium. The nucleolar shell has begun to break down. There is a granule surrounded by a clear area lying on the cytoplasm close to the nuclear membrane.

Figs. 20–22. Stages in the disintegration of the nucleolar shell, and formation of chromatin segments by the condensation of the karyosomes.

Figs. 23, 24. Formation of chromomeres. The plasmatic portion of the nucleolus persists. At × one segment persists.

Fig. 25. A crushed cell showing various stages in the formation of chromomeres. There is one dumb-bell-shaped chromosome fully formed. The plasmosome persists. At × one chromosome is formed.

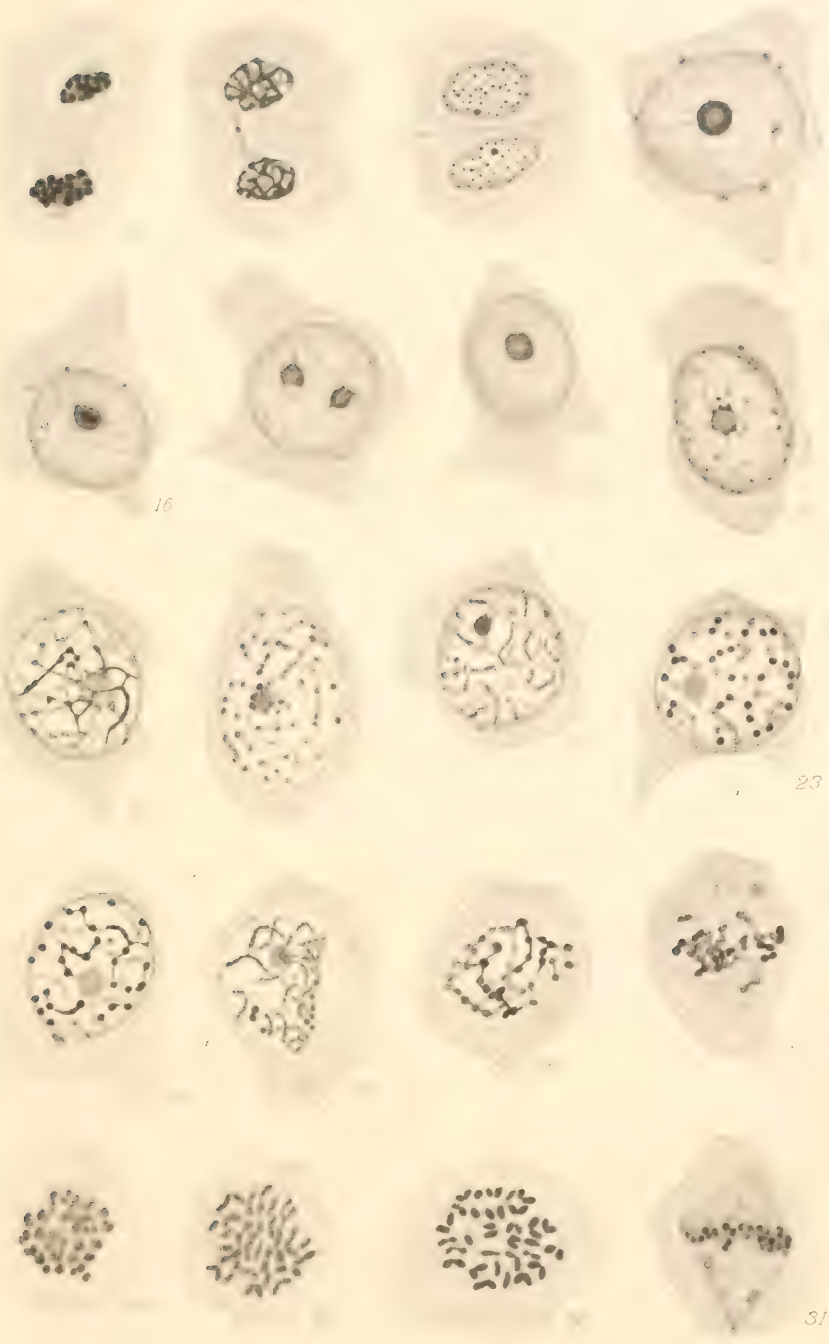
Fig. 26. Late prophase. The nuclear membrane has broken down. There are forty-eight chromomeres connected by linin strands. The plasmosome has disappeared.

Fig. 27. Still later stage, showing the formation of chromosomes by the pairing of the chromomeres.

Figs. 28, 29. Polar views of the metaphase. Many of the chromosomes have split so that many more than twenty-four are visible.

Fig. 30. Metaphase of spermatogonium, polar view. Many of the chromosomes are in the process of splitting.

Fig. 31. Side view of spindle. The chromosomes have begun individually their migration. The centrosomes are minute granules at the poles of the spindle.




H.B.B. del.

## PLATE 3.

All figures are from isolated cells, and are magnified 2600 diameters.

Fig. 32. Anaphase of last spermatogonial division. The cell has been partly crushed so that the chromosomes are separated and show their constricted form.

Fig. 33, *a*. "Resting" stage of spermatogonium.

*b*. Anaphase. Stout interzonal filaments connect the daughter plates.

Fig. 34, *a* and *b*. Each a polar view of the daughter plate of a spermatogonial division.

Fig. 35. Late anaphase. The interzonal filaments are stout. The chromosomes are densely compacted.

Fig. 36. Still later stage. The chromosomes commence to move apart. The centrosomes have disappeared.

Fig. 37. Telophase. The chromosomes have become connected into an irregular network. The interzonal bridge still persists.

Fig. 38. Later telophase. The chromatin networks have commenced to become diffuse.

Fig. 39. Still later stage. The nucleolus is now visible in each daughter cell.

Fig. 40. "Resting" stage of primary spermatocyte. The nucleolus is a homogeneous body. The cytoplasm of the right-hand cell contains a metaplasmic mass.

Fig. 41. "Resting" stage of primary spermatocyte with two nucleoli.

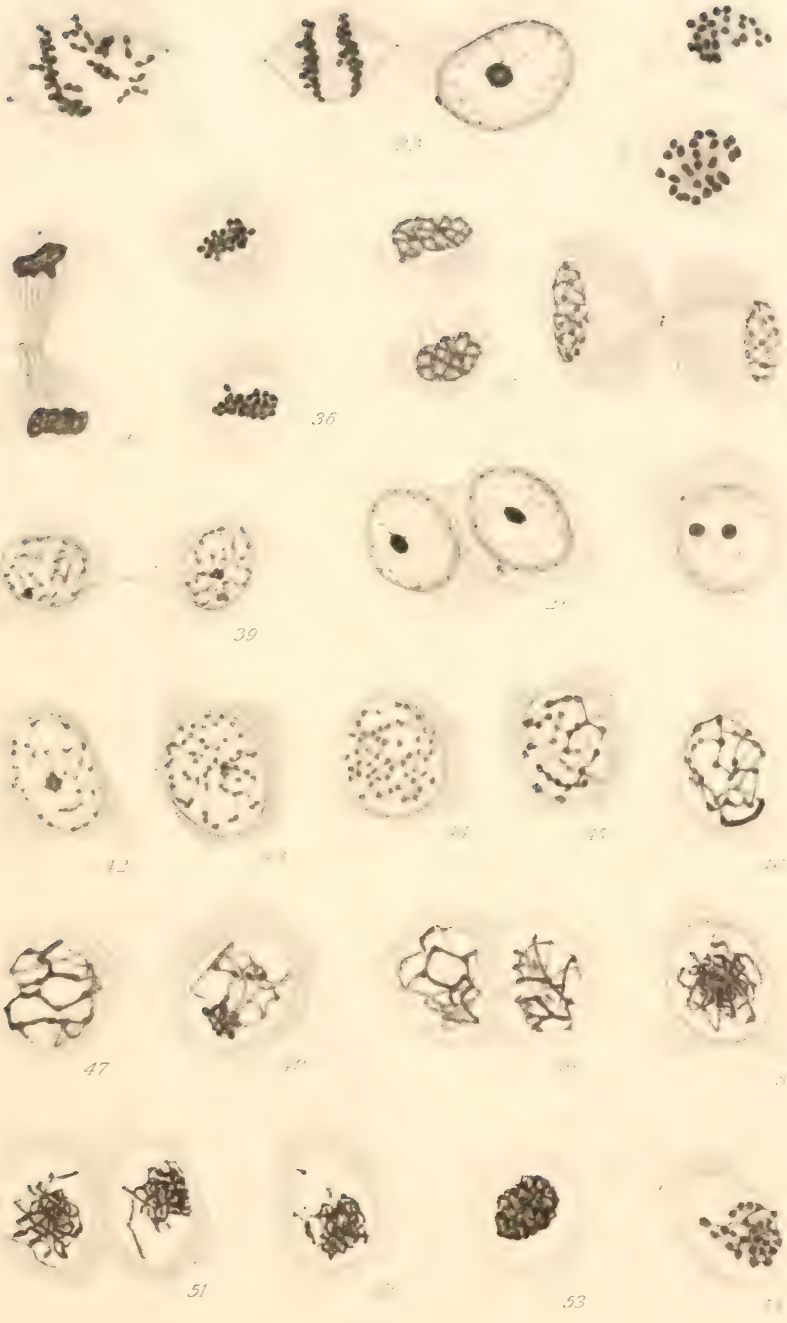
Figs. 42–44. Stages in the prophase of primary spermatocyte. The nucleolus breaks down, and the karyosomes increase in size.

Figs. 45, 46. Later stages. The karyosomes become condensed to form the chromatin reticulum.

Fig. 47. Chromatin segment stage. The strands of the net are dense and homogeneous.

Figs. 48, 49. Chromatin net stage, showing different degrees of contraction.

Figs. 50–54. Various stages in the prophase of the primary spermatocyte, showing contraction phases.



H B B del

## PLATE 4.

All figures are from isolated cells, and are magnified 2600 diameters.

Figs. 55, 56. Late prophase. The chromatin net has segmented into twenty-four separate chromatin bodies. The nuclear contents are somewhat contracted.

Figs. 57, 58. Still later prophase. The nuclear membrane has disappeared. The twenty-four chromomeres are still separate.

Fig. 59. The chromomeres are dense, and have commenced to pair.

Fig. 60. Late prophases. Eight bivalent chromosomes have been formed by the pairing of the chromomeres; six chromomeres are still separate. The linin strands connecting the chromomeres still persist.

Fig. 61. Early metaphase, polar view. There are twelve bivalent chromosomes.

Figs. 62–64. Metaphase of the common type, side view. The chromosomes are dumb-bell-shaped, and are divided by a pulling apart of the thickened ends.

Figs. 65, 66. First maturation spindles of the rarer type. The chromosomes are already divided, and have begun individually their migration toward the poles. They are much larger than spermatogonial chromosomes.

Fig. 67. Oblique view of the two daughter plates of the first maturation division, showing twelve large chromosomes in each.

Fig. 68. Anaphase of first maturation division. The chromosomes are very large and closely crowded. Stout, granular, interzonal filaments connect the two daughter groups.

Fig. 69. Telophase. The chromosomes have become connected into an irregular network.

Fig. 70. Still later telophase. The reticulum is loose. The right-hand cell bears a rudimentary filament on its margin.

Fig. 71. A pair of secondary spermatocytes. The nucleolus has re-formed, in each, and consists of several granules. The interzonal bridge still persists.

Fig. 72. "Resting" stage of secondary spermatocyte. The nucleolus is now a homogeneous structure.

Fig. 73. Prophase of secondary spermatocyte. The nucleolus has broken down, and the karyosomes have increased in size.

Figs. 74–79. Various stages in the prophase of the secondary spermatocyte showing abnormal contraction phases.

Fig. 80. Prophase. Successive stages in the formation of the chromatin reticulum by condensation of the chromomeres.

Fig. 81. Still later prophase. The nuclear membrane is still intact. Twelve chromosomes are now formed.

Figs. 82, 83. Polar views of metaphase of second maturation division, showing twelve chromosomes.

Fig. 84. Metaphase of second maturation division. The centrosomes are prominent.

Fig. 85. Anaphase. The chromosomes are much smaller than in the first maturation division. Stout interzonal filaments connect the daughter groups.

Fig. 86. Late anaphase. The chromosomes are densely massed. The centrosomes persist.

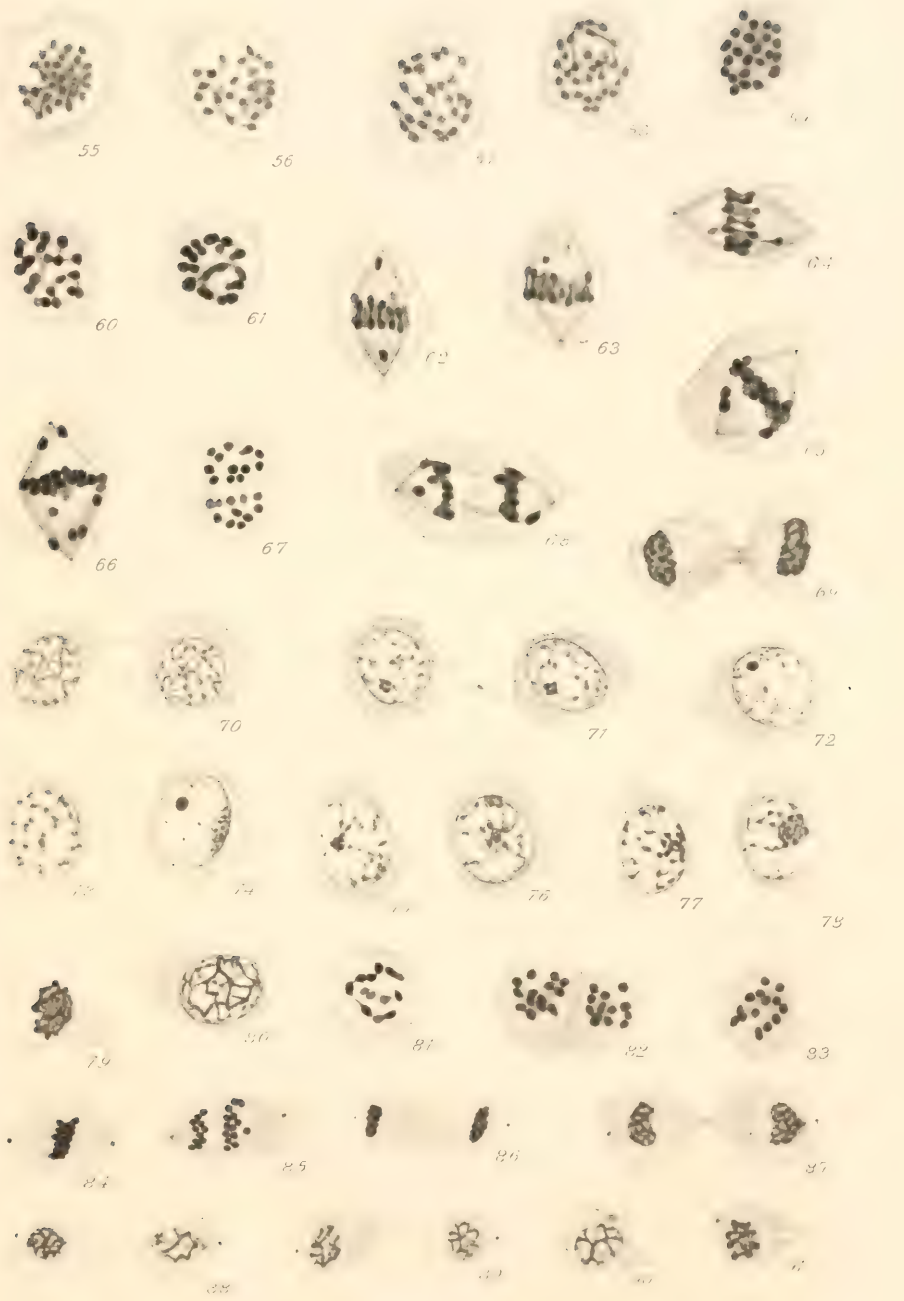
Fig. 87. Telophase. The chromatin forms a dense network. The centrosomes are still visible.

Fig. 88. A pair of daughter spermatids, still connected by the interzonal filaments. The preparation has been crushed, so as to partly separate the filaments. The centrosomes are prominent; and there is a short filament attached to the centrosome of the left-hand cell.

Fig. 89. Pair of spermatids, slightly later stage.

Fig. 90. A spermatid. The interzonal bridge has now broken down, and the polar remnants have become metamorphosed into a spherical archoplasmic mass. The cytoplasm contains several deeply stained granules.

Fig. 91. Spermatid shortly after the breaking down of the interzonal bridge.




HBB del.

## PLATE 5.

All figures are magnified 2600 diameters. Figures 92–108 and 120–123 from isolated cells; figures 109–119 from sections.

Fig. 92. Spermatid. The interzonal remnants have dwindled to a small sphere. The axial filament extends inward from the centrosome.

Fig. 93. Spermatid. The centrosome has divided, and the halves lie in the main axis of the cell. The nucleus has attained its greatest size. The interzonal remnants have disappeared, but there are two archoplasmic masses lying one on either side of the axial filament.

Fig. 94. Spermatid. The inner centrosome has nearly reached the nucleus; it is connected with the outer centrosome by the axial filament. In this cell the interzonal remnants still persist.

Fig. 95. Spermatid. The inner centrosome has reached the nucleus and become flattened against it.

Fig. 96. Metamorphosis of the spermatid. The tail has now grown to a considerable length; the acrosome has appeared at the anterior pole of the nucleus. The chromatin is collected in several masses.

Fig. 97. Slightly later stage. The acrosome is a sphere of archoplasm. The chromatin has become diffused so that the nucleus is uniformly deeply stained. The two archoplasmic masses have grown and occupy most of the space between the nucleus and the posterior margin of the cell.

Fig. 98. The same stage, seen in a plane perpendicular to that of Figure 97. The two archoplasmic masses nearly cover each other, one lying at high, the other at low focus, and the axial filament running between them.

Fig. 99. Later stage. The nucleus has assumed a conical form, and shows a deeply stained basal plate.

Fig. 100. Polar view of this same stage.

Fig. 101. Still later stage. The cytoplasm has become vacuolated.

Fig. 102. Adult spermatozoön. The middle piece consists of three separate archoplasmic masses.

Fig. 103. Giant spermatid with two centrosomes and two tail filaments.

Fig. 104. Giant spermatid with three centrosomes and three tail filaments.

Figs. 105, 106. Later stages in the metamorphosis of giant spermatids, showing evidences of degeneration.

Fig. 107. Multiple spermatozoön with two nuclei, two centrosomes, and two tails.

Fig. 108. Multiple spermatozoön with two nuclei, three centrosomes, and three tails.

Fig. 109. "Resting" stage of oögonium. The nucleolus is compound.

Fig. 110. Early prophase of oögonium. The karyoplasm has disappeared, and the karyosomes increased in size.

Fig. 111. Prophase of oögonium, showing contraction.

Fig. 112. Prophase. The karyosomes have partly condensed to form chromatin segments.

Fig. 113. Prophase. The nucleolar shell has broken down, but chromatin masses are still attached to the margin of the persistent plasmosome.

Fig. 114. Later prophase. The nucleolus has disappeared. The chromatin is condensed into segments of the reticulum.

Fig. 115. Still later prophase. The nuclear membrane has broken down. Several dumb-bell-shaped chromosomes have been formed by the condensation of the chromatin segments.

Fig. 116. Metaphase; polar view. The chromosomes are dumb-bell-shaped; several of them have divided.

Fig. 117. Late metaphase. Most of the chromosomes are in process of splitting.

Fig. 118. Metaphase in side view. The chromosomes appear as spheroidal masses. The centrosomes are now visible.

Fig. 119. Anaphase. The chromosomes form daughter plates. Most of them preserve their orientation parallel to the plane of future cell division. Stout interzonal filaments connect the two daughter groups.

Fig. 120. "Resting" stage of oöcyte. The nucleolus is compound.

Fig. 121. Early pseudoprophase of oöcyte.

Fig. 122. Pseudoprophase. Several chromatin segments have been formed by the coalescence of the karyosomes.

Fig. 123. Later stage of the same. The nucleolar shell has partly broken down.

H B B. del.

## PLATE 6.

Figures 124–130 from isolated cells; figures 131–139 from sections.

Figs. 124–126. Late pseudoprophase. The nucleolar shell has broken down, leaving a persistent plasmosome. The chromatin is entirely condensed into "beaded" segments. × 2600.

Fig. 127. Regression of pseudoprophase. The chromatin segments are irregular and have commenced to break down. × 2600.

Fig. 128. Later stage in regression. The chromatin segments have largely broken down. The plasmosome has grown much larger. × 2600.

Fig. 129. "Resting" stage of oöcyte, following the pseudoprophase. The nucleolus is a homogeneous structure. × 2600.

Figs. 130–138. Growth stage of the oöcyte.

Fig. 130. The chief nucleolus has become vacuolate, and an accessory nucleolus has been formed. The chromatin is finely diffused and has largely lost its staining capacity. × 900.

Fig. 131. Later stage. The chromatin, which has regained its affinity for stains, consists of a large number of angular masses of granules. Both chief and accessory nucleoli, as well as the nucleus as a whole, have increased greatly in size. × 900.

Fig. 132. Still later stage. The chromatin masses have become arranged in strands, many of which are in the form of Y's and V's. × 900.

Fig. 133. Section of late stage in the growth period, showing segments of numerous chromatin strands. The chief nucleolus contains five vacuoles. × 900.

Figs. 134, 135. Chief nucleolus of oöcyte. The ground substance contains deeply stained strands. × 2600.

Fig. 136. Chief nucleolus, containing many deeply stained strands and granules. × 2600.

Fig. 137. Chief nucleolus, ordinary type, containing vacuoles. × 2600.

Fig. 138. Chief nucleolus. The walls of the vacuoles are deeply stained. × 2600.

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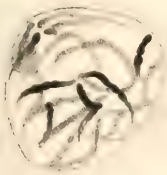

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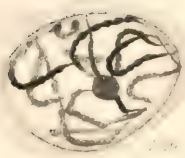

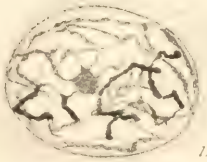

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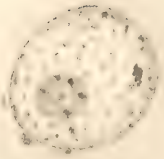

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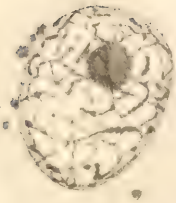

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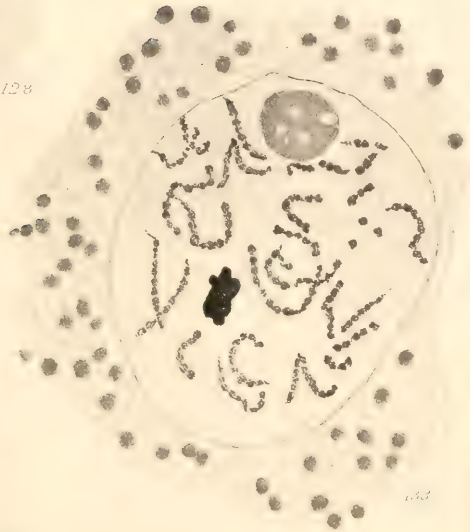

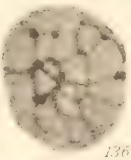

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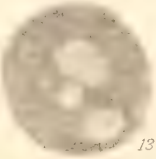

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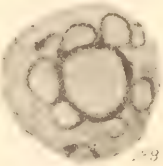

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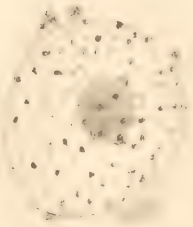

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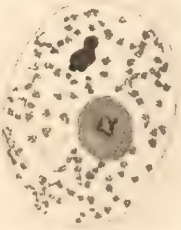

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## PLATE 7.

All figures are from entire eggs stained in borax carmine.

Fig. 139. Fertilization. The egg nucleus is in the metaphase of the second maturation division. The sperm structure consists of head and middle piece, and is surrounded by astral radiations. × 500.

Fig. 140. Fertilization. The egg nucleus is in the anaphase of the second maturation division. The middle piece of the sperm structure has disappeared, and the astral radiations focus at some distance from the sperm nucleus. × 500.

Fig. 141. The sperm aster has divided, and the two resultant asters lie at opposite sides of the sperm nucleus. A supernumerary spermatozoön is attached to the vitelline membrane. The egg nucleus is in the anaphase of the second maturation division. × 500.

Fig. 142. An egg showing polyspermy. Each supernumerary sperm nucleus is accompanied by an aster. One sperm nucleus has joined the egg nucleus, and its two asters lie on either side of the latter. × 500.

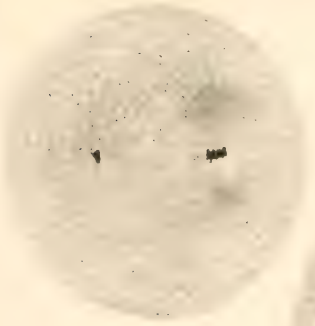
Fig. 143. Union of the germ nuclei. The sperm asters lie in the plane of union. The egg nucleus is in the reticulate "resting" condition. × 1780.

Fig. 144. Union of the germ nuclei. The sperm nucleus, which lies in a clear area, has increased in size, and become more spongy. The sperm asters lie on opposite sides of the egg nucleus. × 1780.

Fig. 145. Later stage of the same. The sperm nucleus forms a cap over part of the egg nucleus, and has become less dense. × 1780.

Fig. 146. Fusion of the germ nuclei. The sperm nucleus has broken up into a large number of small chromatin masses. The egg chromatin forms an irregular threadwork; and both maternal and paternal chromatin are enclosed within a single membrane. × 1780.

Fig. 147. Later stage in fusion. The maternal and paternal chromatin form threadworks which are no longer distinguishable, though the fusion nucleus still has a constricted form. × 1780.



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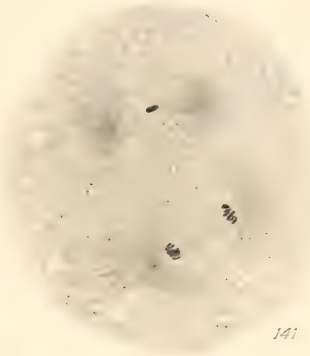

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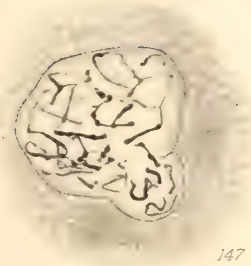

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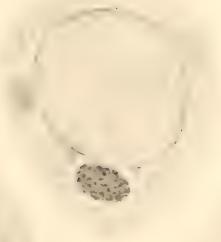

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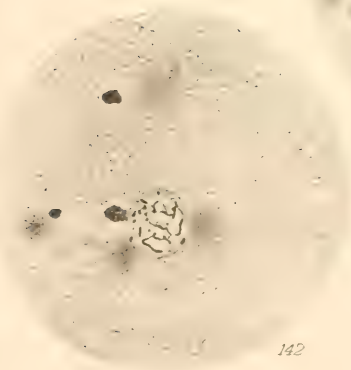

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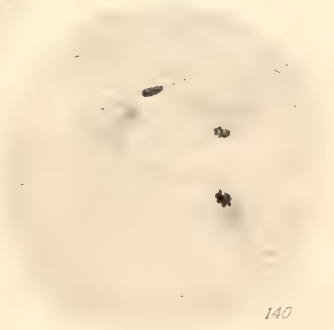

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## PLATE 8.

All figures are from entire eggs stained in borax carmine.

Fig. 148. Union of the germ nuclei by apposition. In the egg nucleus the chromatin forms a threadwork; in the sperm nucleus it consists of many small masses connected with one another by linin strands. × 1780.

Fig. 149. Union of the germ nuclei by apposition. The two nuclei are of nearly equal sizes and are both in the reticulate resting condition. × 1780.

Fig. 150. First cleavage nucleus formed by the fusion of the germ nuclei. The chromatin forms an irregular threadwork, in which the maternal and paternal components are not distinguishable. × 2600.

Fig. 151. Prophase of first cleavage. The nuclear membrane has broken down, and the chromatin forms separate segments. × 2600.

Fig. 152. Late prophase of first cleavage. There are twenty-four chromosomes formed by the condensation of the chromatin segments. Maternal and paternal groups are not distinguishable. × 2600.

Fig. 153. Metaphase of first cleavage spindle. The chromosomes are in the full somatic number. The asters are small. × 1780.

Fig. 154. Oblique polar view of daughter plate of first cleavage, showing the full somatic number of chromosomes. × 2600.

Fig. 155. Metaphase of second cleavage. The chromosomes are in a reduced number. The asters are small. × 1780.

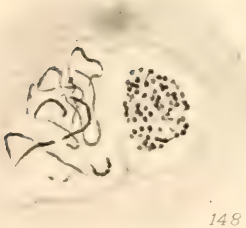
Fig. 156. Anaphase of second cleavage. The asters are much larger, and the daughter chromosome groups form minor centres of radiation. × 1780.

Fig. 157. Polar view of daughter chromosome group of second cleavage. There are twelve bivalent chromosomes, several of which show their double nature by their constricted outlines. × 2600.

Fig. 158. Polar view of daughter chromosome group of second cleavage. There are nine chromosomes which are clearly bivalent; the remaining five are probably univalent. × 2600.

Fig. 159. Side view of fourth cleavage spindle in the metaphase. The chromosomes are present in the full somatic number. × 1780.

Fig. 160. Polar view of daughter chromosome group of fourth cleavage. There are twenty-four chromosomes. × 2600.



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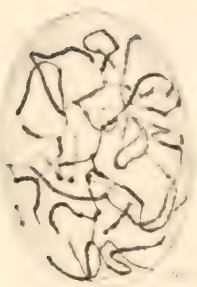

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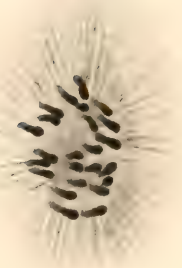

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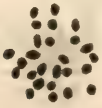

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